Copper Chemistry, Toxicity, and Bioavailability and Its Relationship to Regulation in the Marine Environment

Office of Naval Research Second Workshop Report

A. Zirino, ed.
P. F. Seligman, ed.

SPAWAR
Systems Center
San Diego

SSC San Diego
San Diego, CA 92152-5001

"20030106022"

EXECUTIVE SUMMARY: SECOND WORKSHOP REPORT

Three years after the First Office of Naval Research (ONR) Copper Workshop, the issue of the chemistry, toxicity, and bioavailability of copper (Cu) in the marine environment and its relationship to regulatory standards was reassessed in the course of a second meeting of the Navy user community, government regulators, and scientists. As in the first workshop, the invited participants offered a series of arguments that presented their particular experience and expertise on the issue of copper in an estuarine environment, be it that of a user, scientist, or regulator. Open discussion among all attendees was encouraged. After their presentations, users and scientists met separately to delineate and focus on the most salient issues in their category, with all participants being able to move freely between the two groups.

The workshop concluded with the discussion leaders presenting each group's recommendations to all attendees and with a final discussion of issues by a panel of experts, prompted by questions from the assembly of attendees.

The success of the second workshop may be measured by how closely it came to its original objectives, which were to

- Define Navy copper issues, problems, and requirements

- Define current status and future direction of Cu regulations

- Further the understanding of the relationship between copper speciation, bioavailability, and toxicity

- Determine the state-of the-science of the Cu free ion activity and biotic ligand models

- Develop a consensus on the maturity of free Cu ion activity and complexation capacity measurement capabilities

By almost any standard, the workshop must be considered a success. Navy copper issues were presented at length from both the ships' operational perspective, viz., the maintenance of operationally efficient ships' hulls, to the perspective of shore facilities charged with the application of copper-based coatings and their disposal and with managing both point sources and stormwater (see papers in this workshop report by Cotnoir; Beckwith). The presenters made several trends evident. The first of these is the continuing willingness and efforts by naval facilities to limit the environmental impact of their (unavoidable) copper discharges (e.g., in-water hull cleaning), primarily through various technological improvements (see workshop papers by Bohlander; McCue). These improvements ranged from relatively simple steps such as the filtering of copper particles out of effluents to the much more difficult search for reduced toxicity or nontoxic antifouling coatings (see workshop papers by Haslbeck & Montemarano; Ingle). The second apparent trend is the equal willingness of regulatory agencies to work hand-in-hand with the Navy to reach the common goal of reducing or eliminating copper discharges that are toxic to the environment. The current status and future directions of copper regulations were discussed in at least two workshop papers: Delos and Mitchell discussed the U.S. Environmental Protection Agency (EPA) perspective, and Smith presented ongoing work with a joint Department of Defense/U.S. EPA effort toward developing Uniform National Discharge Standards (UNDS) for military vessels. The U.S. EPA recognizes that site-specific Cu speciation and bioavailability are important for determining environmental effects and intends, to the

extent possible, to incorporate scientific developments in toxicity and bioavailability into its regulatory program.

The main approach, aimed at safeguarding estuarine waters, attempts to continually reduce the concentration of copper entering receiving bodies of water and is followed by both the Navy and the regulatory agencies. While this is undoubtedly a correct approach, as water-quality standards are lowered, a point is reached where it becomes prohibitively expensive, and perhaps even technologically impossible to reduce the copper load reaching the environment. This limitation is well understood, and therefore, regulatory agencies, Navy scientists, and contractors are also following a second approach. The second direction is to attempt to understand the natural mechanisms that serve to protect the environment, such as dilution, complexation, and sedimentation. These processes reduce copper toxicity by reducing the overall copper concentration and by converting a portion of the concentration to a nonreactive or nontoxic state. This process occurs principally via complexation and/or adsorption, viz., the combining of copper with large molecules or particles, either on their surface or internally. Not only does this process reduce toxicity, but it also aids sedimentation, e.g., removal from the water column (see workshop paper by Chadwick et al.). As was pointed out in the First ONR Copper Workshop, the use of available copper in water-quality criteria, rather than the total (dissolved) copper concentration is a scientifically sound approach.

Copper complexation was discussed in at least four workshop papers. Santschi et al.; Gordon; Donat & Burdige; and Rivera–Duarte et al. showed that copper in estuarine environments is complexed to a great extent (>99.9%) by natural ligands, thereby reducing the active concentration of copper in the water column by at least three orders of magnitude, a phenomenon not considered in standard water-quality assessments. The remaining active copper, so termed the copper activity $(Cu(II)_{aq})$, was shown to be toxic to mussel and sand-dollar larvae (see workshop paper by Rivera–Duarte et al.) and inversely proportional to copper concentration in San Diego Bay (see workshop paper by Chadwick et al.), thereby suggesting that the complexing agents were produced in response to copper loading. This observation was confirmed experimentally and presented at the workshop by Santschi et al., and by Gordon, and has been widely confirmed in the literature. Allen et al.; Delos & Mitchell; and Paquin presented and discussed in their papers a method that both calculates the reduction of copper toxicity caused by complexation, (viz., calculates the copper activity), and, simultaneously, calculates the possible effect on the biota. This model, known as the Biotic Ligand Model (BLM), is based on the toxicity of copper deposited on fish gills and has shown considerable promise in freshwater media. It remains to be studied in seawater, but its application in saline media looks feasible. Direct methods of measuring copper activity were also discussed at the second workshop. Rivera–Duarte et al. and De Marco presented works that showed that copper activity and copper complexation could be measured directly in seawater by using ion-selective electrodes. Skrabal, on the other hand, discussed voltammetric methods to accomplish the same purpose. Thompson et al. discussed a novel fluorescence technique for measuring copper activity that was based on copper complexation with carbonic anhydrase. Finally, Chadwick et al. presented a comprehensive, tidally driven estuarine model that accounted for copper dispersion, sedimentation, and complexation. The model was used to accurately calculate the steady-state copper concentration and steady-state copper activity in San Diego Bay, as well as the concentration of copper-binding ligands present in the various parts of the bay.

Perhaps the most important achievement of this Second ONR Copper Workshop is that the participants reached the consensus that copper complexation is a ubiquitous phenomenon, largely produced by the resident microorganisms themselves as a defense against copper toxicity. Logically, then, it is the uncomplexed copper that needs to be monitored to predict toxicity, not the total dissolved copper concentration, a large portion of which is in nonreactive forms. It is this clear fact that seems to have

been ignored by both the regulatory agencies and the Navy user community. At present, the direct use of copper activity as a measure of toxicity has not been "officially" encouraged, nor have any steps been taken to facilitate this task, such as the sponsoring of calibrations and intercomparisons between available techniques. This is most likely because, at present, there is no approved methodology for measuring the active form of copper. However, it appears that the regulatory agencies have understood the concept of activity for some time and have embraced it implicitly in the use of the water-effects-ratio (WER) technique and in their present interest in the BLM. Nevertheless, the lack of a clear and verified methodology for measuring copper activity is delaying progress and probably causing unnecessary expenses for the user community.

The "methods" papers presented at the workshop support the argument that the science of direct copper activity measurements in marine waters is now mature enough to be used in conjunction with the measurement of copper concentration in environmental assessments. With enough data, it may indeed be possible to connect copper activity levels to toxicity for a large number of marine organisms, and provide the user community with a practical, scientifically sound scale for determining the impact of its copper releases on the environment.

EXECUTIVE SUMMARY: FIRST WORKSHOP REPORT

Copper is one of the most ubiquitous contaminants found in many industrial and nonpoint source effluents (including discharges from Navy facilities and ships) into the marine environment. Discharge copper concentrations often exceed existing water-quality criteria (WQC) or standards, both in the effluent and the receiving systems. Regulatory compliance is often challenging because of the many copper sources and the adoption of very conservative water-quality standards. Invited speakers from the Navy user community, regulatory community, Office of Naval Research (ONR)-sponsored researchers, and other prominent scientists in the fields of trace-metal speciation and ecotoxicity participated in the workshop. The goal of the workshop was to improve our scientific understanding of copper in the marine environment and attempt to develop a solid scientific basis for future approaches to copper regulation. Nearly 20 presentations were made over a period of 2 days, at the end of which the "user-regulator" speakers and the scientific speakers met in two working groups to draft a set of recommendations. The general conclusions of the workshop were as follows:

The legal release and disposal of copper or copper-laden effluents into estuarine waters poses a serious environmental, engineering, and economic challenge to the Navy and the country in general. The challenge is due to the fact that the U.S. Environmental Protection Agency (EPA)-mandated WQC for copper effluents are at, or very close to, the ambient or "normal" concentrations of copper in many estuaries, which are at the low μg/L level. The treatment of copper-contaminated effluents to achieve such low levels, even if they are technologically attainable, may be cost-prohibitive, and may not be warranted, since there is little, if any, direct evidence in the natural environment of toxicity being caused by small increases in copper levels.

The present EPA-mandated WQC, justifiably fulfill their mission of protecting the environment, but do so from a relatively weak scientific basis and, most probably, are over-protective. A large body of scientific data indicates that it is the concentration of the "free" or aqueous cupric-ion species $(Cu(II)_{aq})$ that relates best, but not exclusively, to the toxicity of marine organisms. However, present WQC are based on concentrations of "total recoverable" copper and on unrealistic laboratory tests. The discrepancy between the current scientific knowledge and WQ standards tends to undermine confidence in the general applicability of the latter. The questions of copper speciation and bioavailability are being addressed by the regulatory community indirectly via the adaptation of a number of mechanisms, such as the use of dissolved copper concentrations rather than total recoverable copper and the use of Water Effects Ratios (WERs). However, these measures are, at present, an unsatisfactory substitute for realistic WQC/Standards based on bioavailability. Nevertheless, it's fully recognized by the workshop participants that the issues of speciation, bioavailability, and environmental impact of copper in estuaries and coastal waters are highly complex and will require much further study before they are satisfactorily resolved. At present, these studies should concentrate on (1) the development of standardized analytical methods of sampling and analysis, (2) the study of in situ physical and biological process that may affect copper speciation, and (3) how toxicity effects on individual organisms ultimately translate into population and community effects.

CONTENTS

Figures

Tables

WORKSHOP GOALS

Similar to the first workshop, the goal of the second workshop was to clarify our scientific under-standing of the nature, toxicity, and fate of copper (Cu) in the marine environment so as to develop a better basis for future approaches to copper regulation. The specific objectives of the second work-shop were to bring Navy and regulatory representatives and scientific experts together to (1) provide an update of the current status and future direction of Cu regulations, (2) re-examine problems and issues associated with the introduction of copper into the estuarine environment, (3) discuss anew the relationship between copper speciation, bioavailability, and toxicity in the light of recent research results and data, and (4) gauge the feasibility of modifying or changing existing concentration-based regulations in view of the analytical Cu-speciation and complexation-capacity techniques currently available.

WORKSHOP ORGANIZATION

The second workshop took place at the Officer's Club at the Naval Academy in Annapolis, MD, 1 to 2 November 2000. Similar to the first workshop, the second workshop consisted of a series of individual presentations by invited speakers from the Navy user community, internationally known scientific experts (most from the U.S.), and representatives from the U.S. Environmental Protection Agency (EPA). The audience consisted of all the speakers plus additional attendees from the Navy community, universities, and the U.S. EPA. The total number of attendees was once again kept small to promote discussion, and open debate was encouraged. On the morning of 1 November, introductory comments by Dr. Stephen McElvany and Alex Lardis of the Office of Naval Research (ONR), were followed by a presentation on the "regulatory perspective" by C. Delos and J. Mitchell of the U.S. EPA. The "user" community then presented eight works describing Navy copper issues of importance to both shore facilities and ships.

These presentations were then followed by 14 scientific presentations that extended over the 2 days of the workshop, covering (1) water-quality criteria (WQC) and copper speciation, (2) bioavailability, (3) biological response, (4) predictive models, and (5) methods of analysis.

As in the first workshop, the presentations and their discussions lasted until noon of the second day. After a recess, all the workshop participants were re-convened into either of two groups. Group (1) consisted of Navy users and regulators; group (2) was composed principally of scientists. The two groups met separately to draft a set of recommendations. It was possible to switch from one group to another at will. At the end of these sessions, recommendations were presented to all the attendees by Peter Seligman and Al Zirino of the Space and Naval Warfare Systems Center, San Diego (SSC San Diego) for final discussion. At the end of the workshop, copies of visual-aid materials (transparencies or viewgraphs) were collected from the individual speakers to facilitate the writing of this report. The final report was prepared from this material, individual notes, and from material in the open scientific literature. The workshop program agenda is provided in the next section.

WORKSHOP AGENDA

ONR Second Workshop on Copper Chemistry, Toxicity, and Bioavailability and Its Relationship to Regulation in the Marine Environment

Naval Academy, Annapolis, Officers Club
1 to 2 November 2000

Wednesday, November 1

7:30–8:30	Registration and Continental Breakfast	
8:30–8:40	ONR Introductory Remarks	S. McElvany, A. Lardis
8:40–8:55	Meeting Objectives/Summary of First Copper Workshop	P. Seligman, A. Zirino

Regulatory Perspective

8:55–9:15	Status of National Copper Water-Quality Regulations and Guidance	C. Delos, J. Mitchell

Navy Shore Facilities Copper Issues

9:15–9:45	Regulatory Implications of Copper Criteria and Navy Discharge Permits	D. Cotnoir
9:45–10:00	Break	
10:00–10:20	Shipyard Copper Compliance Issues: Puget Sound Naval Shipyard Case Study	B. Beckwith

Navy Ship Copper Issues

10:20–10:40	Uniform National Discharge Standards: Status and Future	G. Smith
10:40–11:00	Antifouling Coatings Current and Future	M. Ingle
11:00–11:15	Characterization of Coatings for Fouling Control	L. Haslbeck, J. Montemarano
11:15–11:35	In-water Hull Cleaning: Sources and Solutions	J. Bohlander
11:35–11:50	The Future of Underwater Hull Cleaning	T. McCue
11:50–12:05	Navy Copper Sources and Loading	P. Seligman
12:05–1:00	Lunch (on site)	

Scientific Issues

1:00–1:15	Scientific Review of First Copper Workshop	A. Zirino
1:15–1:50	Water-Quality Criteria/Cu Concentrations/Cu Activity	H. Allen

Effects

1:50–2:25	Free Copper Ion Activity, Complexation Capacity and Toxicity in San Diego Bay Waters	I. Rivera
2:25–3:00	Wetland Plants' Roles in Uptake and Transport of Heavy Metals	P. Weis
3:00–3:15	Break	

Speciation

3:15–3:50	Chemical Speciation and Measurement of Cu in Seawater	K. Bruland

Defenses

3:50–4:25	Interactions of Biopolymers (e.g., acid polysaccharides and thiols) with Cu and Other Heavy Metals in Galveston Bay, Texas	P. Santschi
4:25–5:00	Recent Advances that Pose New Problems for Regulators	J. Moffett

Adjourn

Thursday November 2

Defenses (continued)

8:00–8:35	Production of Copper-Complexing Ligands by Bacteria in Culture and by Intact Microbial Communities In Situ	A. Gordon
8:35–9:10	Copper Complexation, Speciation, and Sediment-Water Cycling in a Major U.S. Harbor (Elizabeth River, VA)	J. Donat

Models

9:10–9:45	Use of the Biotic Ligand Model (BLM) in a Model of Survival Time for Fish	P. Paquin
9:45–10:00	Break	
10:00–10:35	Modeling Conservative and Non-Conservative Behavior of Copper in San Diego Bay	B. Chadwick

Analysis

10:35–11:10	Cu Activity and Cu Ligands by Voltammetry	S. Skrabal, M. Shaafer, A. Zirino
11:10–11-45	Fluorescence-based Biosensing of Cu(II) in Seawater	R. Thompson
11:45–12:20	Activity Measurements with Ion Selective Electrodes	R. De Marco
12:20–1:20	Lunch (on site)	

Discussion Groups/Panel

1:20–2:45   Navy User and Regulatory Group I: Summary of Issues, Needs, Regulatory Status and Development of Needs and Recommendations

Scientific Group II: Summary of State of the Science, Data Gaps, and Recommendations

2:45–3:00   Break

3:00–4:45   Combined Panel: Navy User and Regulatory Group Summary; Science Status and Data Gaps Summary; and Panel Discussion and Recommendations

Adjourn

SUMMARY OF PRESENTATIONS

INTRODUCTIONS

Dr. Stephen W. McElvany, Program Manager, Physical Sciences S&T, ONR 331 and Alex Lardis, manager of the Environmental Quality Applied Research Program at ONR provided introductory remarks.

REGULATORY OVERVIEW AND NAVY USER GROUP PRESENTATION

WORKSHOP OBJECTIVES AND SUMMARY OF FIRST COPPER WORKSHOP
(P. F. Seligman and A. Zirino)

The goal of the Second Cu Workshop was to improve scientific understanding of copper speciation, complexation, and toxicity in order to develop the foundation on which to base further improved approaches to Cu regulation. By bringing Navy, regulatory, and scientific representatives together to define problems and issues and evaluate the state-of-the-science, it was hoped that the following could be accomplished:

- Define Navy copper compliance issues, problems, and requirements

- Define current status and future direction of Cu regulations

- Further the understanding of the relationship between copper speciation, bioavailability, and toxicity

- Determine the state-of-the-science of the Cu free ion activity and biotic ligand models

- Develop a consensus on the maturity of free Cu ion activity and complexation capacity measurement capabilities

Navy User Issues, Needs, and Regulatory Status

During the First Workshop in 1997, Navy representatives discussed Cu loading and discharges of concern. Subjects included drydock and other point-source discharges (that periodically exceed permitted limits), and the increasing attention to stormwater contamination. Loading estimates from Navy harbors suggested that antifouling (AF) coatings were often the single largest contributor of copper in those harbors. While in-water hull cleaning of naval vessels represented a small percentage of the total loading (typically less than 1%), it does constitute a high concentration effluent with low frequency and can contribute to copper loading to the sediments.

Some significant regulatory issues were discussed during the meeting. The form of regulated Cu (total or dissolved) is often not related to effects on biota. Copper ion $Cu(II)_{aq}$ is believed to be the most available and toxic species; total recoverable copper (CuT) is not highly relevant to environmental effects. WQC and standards based on total or dissolved concentrations tend to be overly conservative and often result in National Pollutant Discharge Elimination System (NPDES) permit limits that are conservative and very costly to attain. Total Maximum Daily Loading/Wasteload Allocation (TMDL/WLA) calculations mandated by the Clean Water Act have the potential to severely limit discharges if conservative water-quality standards are applied and no consideration of speciation and complexation are taken into consideration. Sediment criteria based on bulk chemistry may also be highly restrictive. The Uniform National Discharge Standards (UNDS) will ultimately

be the guiding regulation for setting all ship discharges, including biocide release from antifouling hull coatings.

A number of pollution-control measures were discussed to reduce copper discharges. Both the Navy and industry need faster and cheaper Cu removal technology. New in-water, hull-cleaning and treatment technology is needed to reduce Cu discharge. Improved measurements and treatment capability are needed at Industrial Waste Treatment Plants (IWTPs) and for drydock wash water, over-spray, and runoff. Faster regulatory acceptance is needed for measurement and pollution-control systems to support scientifically sound standards at achievable cost. The question was asked: "How can science help?" Several suggestions were made on key scientific issues that need to be addressed: (1) Acceptable and relevant measurement tools are needed to support development of appropriate and reasonable water-quality objectives and for defining shipboard marine pollution-control-device performance standards; (2) the time scales of Cu complexation need to be determined and related to toxicity criteria; and (3) nontoxic or reduced release-rate coatings should be developed.

Science Group State of the Art and Data Gaps

A large body of scientific data indicates that it is the concentration of the free or aqueous cupric-ion species ($Cu(II)_{aq}$) that best relates, but not exclusively, to the toxicity of marine organisms. There is a good scientific basis for this, and it goes beyond the notion of a small quantity of copper ($<1\%$ of total Cu) being very toxic. The activity or concentration of $Cu(II)_{aq}$ is a good measure of the lability and mobility of all the copper in the system and, therefore, would be expected to be highly correlated to its uptake by organisms and to its ultimate toxicity.

The current trends of WQC for metals indirectly reflect current knowledge about the importance of speciation through a number of mechanisms, such as the adoption of dissolved copper concentrations rather than total recoverable copper for the WQC. However, many permits still require total measurements. WER is another option that accounts, in part, for the $Cu(II)_{aq}$ sequestering capacity of dissolved organic and colloidal materials found in natural waters. Yet, such measures do not take into account the temporal dynamics of $Cu(II)_{aq}$ and cannot be substituted for the direct knowledge of the dynamics of $Cu(II)_{aq}$.

Evidence provided during the workshop and in the literature suggest that the ratio of free copper to total dissolved copper ($Cu(II)_{aq}/Cu_T$) may vary both temporally and spatially due to local mixing processes, adsorption, or particulates, and the ability of marine microorganisms to produce copper-sequestering material. It is likely that the conditions under which standard toxicity tests are performed do not reflect accurately the $Cu(II)_{aq}/Cu_T$ ratio found in the natural environment. Therefore, in order to better relate WQC to natural conditions, a concerted effort should be made to understand how $Cu(II)_{aq}/Cu_T$ changes over time during toxicity tests as well as in the environment.

Analytical techniques for the measurement of (at least) two of the many possible copper species, $Cu(II)_{aq}$ and Cu_T should be standardized, and trace-metal clean techniques should be used in both sampling and measurement so as to provide unambiguous reference levels for the user community.

REGULATORY PERSPECTIVE

STATUS OF NATIONAL COPPER WATER-QUALITY REGULATIONS AND GUIDANCE
(C. Delos and J. Mitchell)

The goal of this presentation is to describe current progress in the metals water-quality program and to provide its intended near-term direction. To the extent possible, the U.S. EPA intends to incorporate scientific developments in metals toxicity and bioavailability into its regulatory program. It has long been recognized that site-specific speciation and bioavailability are important to determining environmental effects, but the science and modeling capability did not exist to follow up on this knowledge.

Since the last ONR workshop, the following new matters of interest have unfolded at the U.S. EPA:

1. Biotic Ligand Model (BLM). The BLM underwent Science Advisory Board (SAB) review. The review was generally favorable. The SAB made a number of recommendations for additional research to broaden and deepen the model's applicability. Specifically, the SAB made the following recommendations:

- More research efforts be directed toward the prediction of chronic toxicity.

- BLM predictions be further compared to WER results, and the supporting database be broadened to include greater taxonomic and functional diversity.

- Our mechanistic understanding of the BLM be improved.

- A better understanding of the effect of dissolved organic matter (DOM) on metals complexation be obtained. Specific guidance for the model's use be developed.

Some of these recommendations have been taken on directly by the U.S. EPA. Others are part of a longer term research agenda. Short-term efforts are being directed toward incrementally improving the BLM's response to differing values of pH. Nevertheless, there is consensus that the current model, being a substantial improvement over the empirical hardness-based approach the U.S. EPA has been using for the last 20 years, is ready for regulatory application.

Figure 1 shows a comparison of the relative ability of the model to explain differences in LC50s from replicate tests with selected species under different conditions. In part due to the water chemistry differences, and in part due to other factors that contribute to the imprecision of toxicity tests, the variability among unnormalized LC50s is substantial. Normalizing for hardness differences usually (but not always) reduces the variability within a species. Normalizing using the BLM usually (but not always) reduces variability more than the empirical hardness formula. It should be noted that even if the model were perfect and the test conditions precisely known, there would remain significant variability among LC50s for a species due to other factors that yield imprecision in such tests.
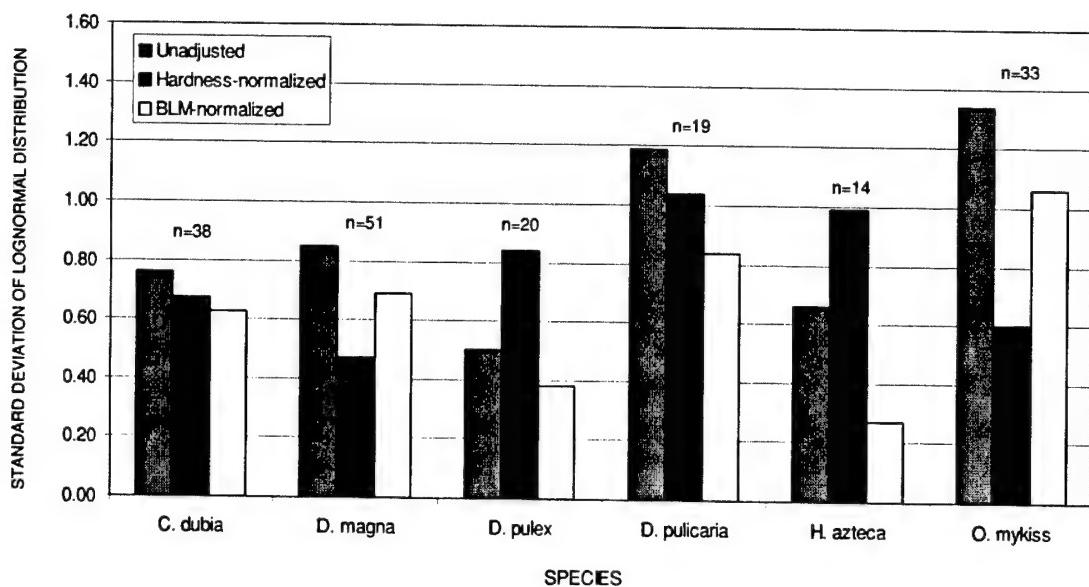
Figure 1. Comparison of intra-species variability using different normalization techniques.

Given the intention to incorporate the BLM into the regulatory program, such application may take two forms: (a) an alternative to the WER (the site criterion-adjustment factor conventionally obtained by toxicity testing using site water) or (b) direct incorporation of the BLM in a newly derived copper criterion. The U.S. EPA's preference is to incorporate it directly into the criterion, and work toward this end is ongoing.

Obtained by literature search, the database of acute toxicity tests suitable for supporting a freshwater copper criterion now includes some 430 measurements with approximately 40 freshwater genera. In the past, these tests would be normalized for water-chemistry differences based only on their measured hardness, while using a hardness relationship empirically estimated from data for several species. Now, however, we are attempting to use the BLM to normalize for multiple differences in water parameters, not all of which were necessarily measured in all the tests. Of the five key model parameters (pH, hardness, alkalinity, sodium, and dissolved organic carbon [DOC]), DOC is the one most likely not to have been measured during the tests. To the extent possible, these types of data gaps are being reduced by contacting the original investigators and by applying reported values from other studies and using similar dilution waters. Uncertainty in the test concentrations of the model parameters is one of the factors contributing to the unexplained variation in the BLM-normalized LC50s shown in Figure 1.

Current activities are also focusing on the development of a user-friendly computer interface, with appropriate documentation, and on investigating the implementation issues associated with the time-variability of water chemistry that would occur at each site of application.

To complete the work on freshwater expeditiously, work on saltwater has been postponed. However, preliminary explorations suggest that the model might perform well in saltwater. There is also interest in extending the model to predict sodium concentrations internal to aquatic organisms. It is thought that this may improve the model's response to external sodium concentrations, while providing a mechanistic connection to the organism's cause of death. Work to confirm the model's

12

applicability to saltwater could begin once the freshwater work is completed, if there were interest among saltwater stakeholders in such a development.

2. An updated aquatic-life-toxicity literature review. To obtain an updated complete review of the literature on copper toxicity, a 1998 *draft* freshwater and saltwater criteria document has been produced. It is currently drafted as a conventional criteria document, with an empirical hardness normalization for freshwater and no water-quality normalization for saltwater. However, our preference would be to incorporate the BLM into the criterion. The value of the draft document is for its literature review. The saltwater criterion derived in the draft is close to the current criterion. The 1998 draft has no official standing and is *not* a proposed criterion. A draft for peer review and public comment is expected in 2002.

3. The streamlining of WER procedure. The WER is an empirical toxicity-testing approach for obtaining criteria adjusted for site-specific bioavailability. A more streamlined alternative to the current cumbersome WER study procedure was released in March 2001. With modification, the streamlined procedure could be applicable to single-discharger saltwater situations.

4. Criteria for averaging period and allowable exceedance frequency. This is not strictly a copper issue but affects all other pollutants as well. In its most recent standards promulgation (the California Toxics Rule), the U.S. EPA chose not to set an averaging period (previously 1 hour) for metals acute criteria. For the chronic averaging period and for the allowable exceedance frequency, the U.S. EPA specified the customary 4 days, and once in 3 years, respectively. However, in response to problematic public comments, the U.S. EPA chose to allow the State the option to derive and use a different averaging period and allowable frequency, with U.S. EPA approval.

Modifications of the averaging period or allowable exceedance frequency can affect pollution-control decisions. In most cases, however, such modifications are not expected to be quite as important as the target criterion concentration.

5. New sediment guidelines. The Equilibrium Partitioning Sediment Benchmarks document for a mixture of cadmium (Cd), Cu, lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn) is complete. However, for policy reasons, the Office of Water prefers not publishing these documents as official guidance. Instead, the current plan is for the Office of Research and Development to publish the documents as technical information.

The acid volatile sulfides (AVS) benchmark is that the sum of the simultaneously extracted metals (SEM) be less than AVS (ΣSEM < AVS). The interstitial water benchmark is that the sum of the toxic equivalents of dissolved metal be less than 1.0 (Σ[M/FCV] < 1.0), where M is the metals concentration and FCV is the conventional chronic criterion for the metal. These benchmarks are recommended to be applied in conjunction with other lines of toxicity evidence. Exceedance of these values is not definitive evidence of a toxicity problem.

Conclusion

Regarding the regulation of copper in marine environments, the most significant items are the completion of technical work on the development of sediment quality guidelines, the expected year 2002 publication of a new draft saltwater copper (water-column) criterion, and steady progress in the development of the BLM, including encouraging indications that it may be adaptable to saltwater. In spite of a commitment to apply the BLM to freshwater copper, the U.S. EPA remains receptive to other feasible approaches for determining or predicting copper bioavailability in saltwater.

NAVY SHORE FACILITIES COPPER ISSUES

REGULATORY IMPLICATIONS OF COPPER CRITERIA AND NAVY DISCHARGE PERMITS
(D. Cotnoir)

WQC to protect aquatic life for copper have presented many compliance challenges for Navy shore facilities. It is well established that concentrations of copper in urban runoff routinely exceed WQC. Such results have caused compliance challenges to many Navy facilities. The Navy facilities generally hold NPDES permits for stormwater discharges and have been required, in many cases, to monitor for copper to determine whether there is reasonable potential to cause or contribute to the impairment of adjacent receiving waters. Efforts have been undertaken in the Norfolk area to establish site-specific WQC for copper to prevent establishment of numeric effluent limits and to facilitate compliance if such limits are developed (Figure 2).
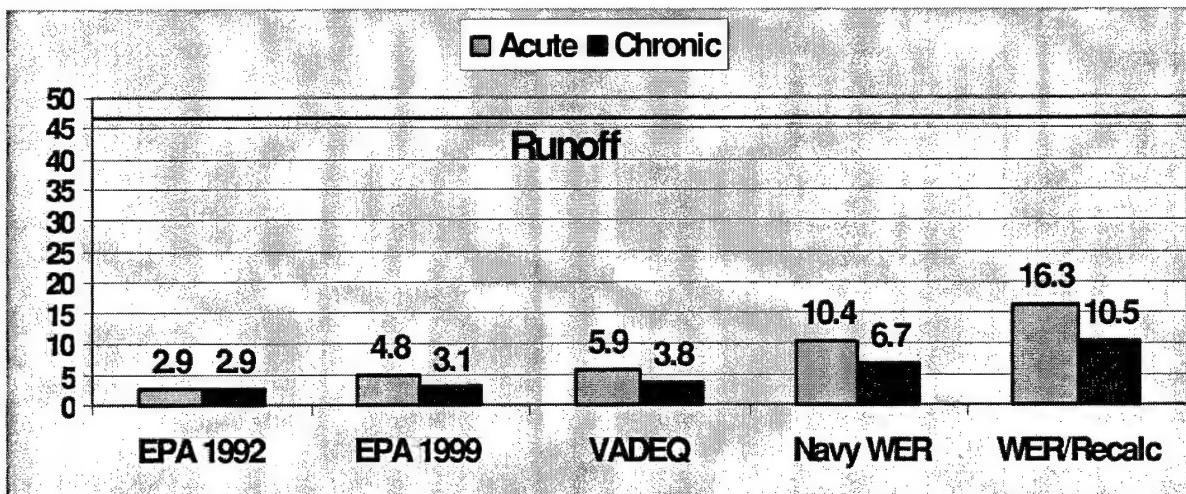
Figure 2. WQC for copper: U.S. EPA, Virginia, and site-specific criteria for Hampton Roads Estuary. (VADEQ stands for Virginia Department of Environmental Quality.)

Despite these efforts, current permits require monitoring for copper with source investigations if data exceed predetermined selection criteria derived from the acute WQC. In the Washington, D.C. area, numeric water-quality-based effluent limits have been developed for copper in stormwater discharges. Efforts are underway to determine source(s) of copper and to establish mixing zones to determine the appropriateness of the specified effluent limits. In the Norfolk area, the Navy has developed a WER for copper. The WER effectively adjusts statewide acute and chronic criteria to site-specific criteria. WERs are costly but generally advantageous to the permittee because sites may vary considerably in their ability to reduce (complex) bioavailable (toxic) copper. WERs can be developed for a single discharge or for an entire region/water body by sampling several sites. In addition to WERs, the Navy has used the recalculation procedure to calculate a site-specific criterion. This recalculation procedure permits the use of toxicity data using local organisms that may be less sensitive to copper.

Copper WQC have also caused copper compliance problems with wastewater discharges to a publicly owned treatment works in the Norfolk area. These problems were caused by high copper concentrations in ships' discharges (from the corrosion of waste piping) that exceeded local limits

established by the publicly owned treatment works. The limits were established to ensure that the discharges would be in compliance with permit limits protective of water quality. The issue was solved by reclassifying the source of copper as a special category of domestic waste rather than as an industrial source. The same problem and solution may be applicable to other Navy sites. These and other compliance issues have and will continue to present unique challenges to the Navy and others because the potential cost of treating stormwater and ship sewage is enormous. Therefore, it is very important that WQC be based on the best science available and that scientific and engineering resources be committed to the development of innovative methods of reducing copper discharges.

SHIPYARD COPPER COMPLIANCE ISSUES: PUGET SOUND NAVAL SHIPYARD CASE STUDY
(B. Beckwith)

Puget Sound Naval Shipyard (PSNS) is located adjacent to the City of Bremerton in western Washington. PSNS is the Pacific Northwest's largest Naval Shore Facility and one of Washington's largest industrial installations. The Shipyard (and the adjacent Naval Base Bremerton) encompasses 353 acres of land, 360 buildings, six drydocks, and nine piers with more than 2 miles of deep-water space. The current mission of PSNS includes performing overhaul, repair, recycling, and engineering design work for ships Navy-wide. Additionally, PSNS serves as a homeport for several ships. These activities generate wastewater such as bilgewater, stormwater runoff, and industrial wastewater subject to federal, state, and local regulatory requirements.

PSNS is a "good neighbor" in the sense that it is committed to serve national needs and also be protective of the local environment. This commitment is carried out via a system of regulations based on the best scientific knowledge available (Figure 3). Despite PSNS' desire to protect the environment, the actual outcome is dependent on the interaction of science, regulation, and compliance. A breakdown in any of these can cause the expenditure of large amounts of resources without achieving the desired results. PSNS' experiences in trying to achieve compliance with limits on the discharges from its drydocks demonstrates the efforts required of naval installations in attempting to achieve and maintain compliance with environmental regulations.
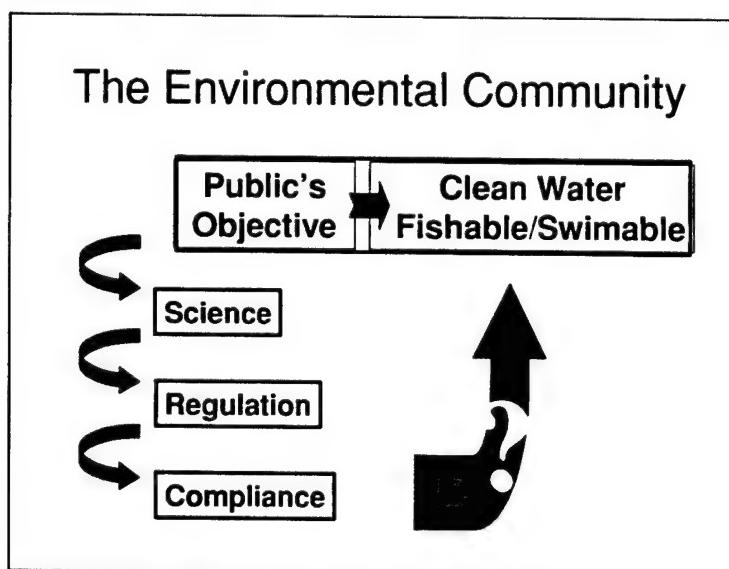
Figure 3. Relationship between environmental objectives and compliance.

PSNS' discharges to Sinclair Inlet are permitted by an NPDES permit issued by the U.S. EPA, Region 10. The current permit was issued in April 1994 and includes stormwater, wastewater from PSNS' steam plant, and discharges from the Shipyard's six dry docks. Shortly after this permit was issued, the Shipyard realized that it could not consistently comply with the limits of 33 µg/l daily maximum (19 µg/l monthly average) for total recoverable copper from the drydock drainage systems (Figure 4).
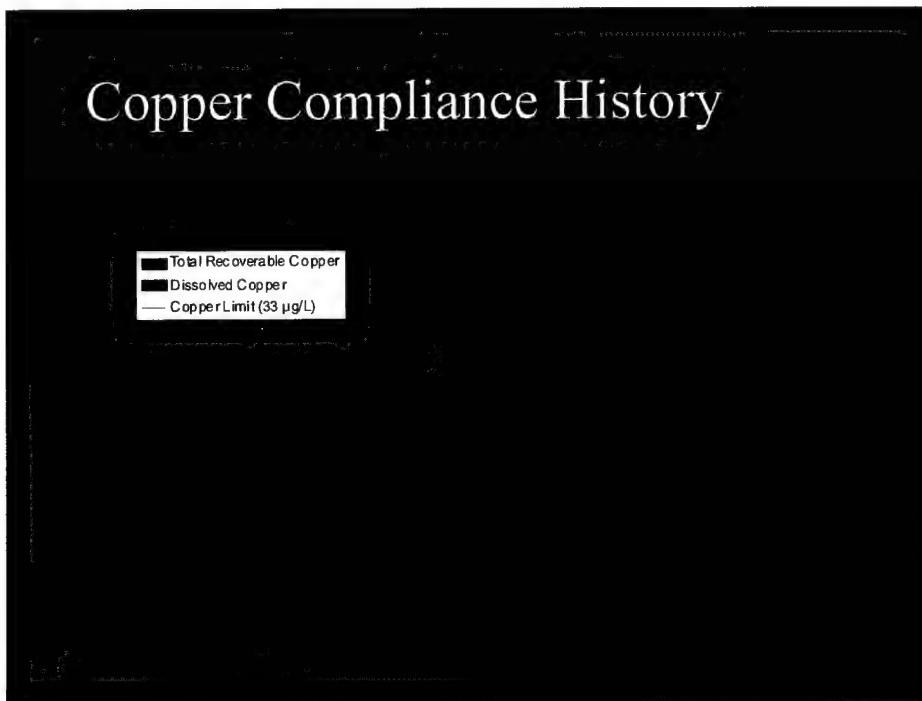
Figure 4. Compliance history for drydocks 1 to 5 at PSNSY (1999–2000).

In May 1999, the U.S. EPA issued the Shipyard a Notice of Violation for the continuing exceedances of the copper limits in water discharged from the Shipyard's drydocks. In February 2000, the U.S. EPA and PSNS signed a Federal Facilities Compliance Agreement (FFCA) that included the steps the Shipyard agreed to take in its efforts to achieve compliance. The FFCA defines compliance as 6 consecutive months without a copper exceedance. It appears that the Shipyard will be able to achieve this goal in the short term; however, long-term compliance will be difficult since the intensity and type of production work fluctuates.

For the past 6 years, the Shipyard has been studying discharges from drydocks and implementing methods for reducing the amount of copper discharged. These methods include changing production processes, installing stormwater collection systems, improving the methods for removing accumulated industrial debris, and isolating the impact of bay silt from the Shipyard's compliance monitoring.

Many exceedances involving high copper concentrations of effluent waters actually involve copper on particles, and considerable effort has been made to remove these particles by filtration. Figure 5 shows the relationship between total copper and dissolved copper prior to filtration. A better understanding of the relative impact of the various forms of copper by the scientific community could result in less stringent regulations on shipyard activities.
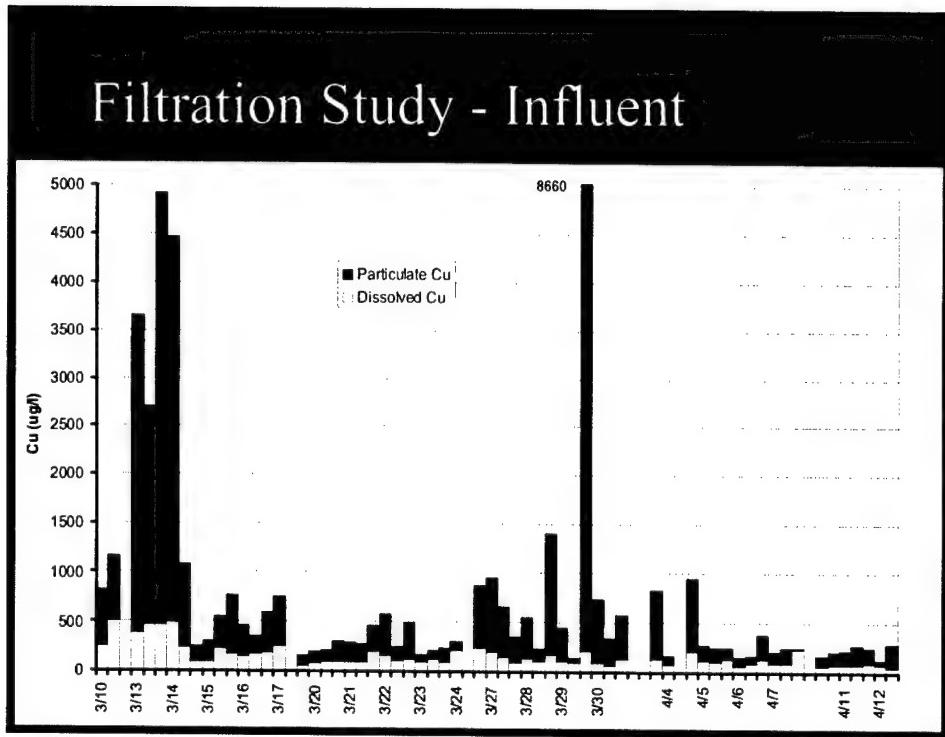
Figure 5. Relationship between dissolved and particulate copper in drydock discharge.

In addition to the difficulty of complying with the Shipyard's current copper limits, it is unknown what limits will be in the Shipyard's next permit. If the limits in the next permit are based on the same rationale as the current permit, it is likely that the new limits will be significantly lower, based on recent measurements of ambient water quality. The U.S. EPA, Region 10 recognizes the difficulties facing the Shipyard and has agreed that it may be necessary to revise the scheme by which PSNS is regulated for copper.

PSNS has spent millions of dollars in modifying its drydocks and continues to spend millions of dollars yearly on water treatment and best management practices. Despite these efforts, the Shipyard still cannot guarantee continued compliance with the current regulations, and future regulations may be more restrictive. Further reductions in copper discharges will require expenditures far in excess of the current spending. To justify this expense, the regulations must be based on the best available science to ensure that the regulations achieve the desired results.

NAVY SHIP COPPER ISSUES

UNIFORM NATIONAL DISCHARGE STANDARDS (UNDS): STATUS AND FUTURE (G. Smith)

This presentation provides an overview of Department of Defense (DoD) and U.S. EPA efforts to (1) establish a system of Uniform National Discharge Standards (UNDS) for liquid discharges from vessels of the U.S. Armed Forces and (2) establish performance standards for marine pollution-control devices (MPCDs) deployed to control those discharges. Congress established UNDS through legislation placed in section 325 of the 1996 National Defense Authorization Act. This legislation requires (1) DoD and the U.S. EPA to identify and evaluate incidental discharges from Armed Forces vessels to determine which discharges would be potentially harmful to the environment and require control; (2) authorizes DoD and the U.S. EPA to establish MPCD standards for those discharges that require control; and (3) UNDS provides the states, working in conjunction with the U.S. EPA, with the ability to establish no-discharge zones for one or more discharges. UNDS defines a discharge as virtually any point or nonpoint discharge (except sewage and solid waste) into the water column incidental to normal operation, repair, testing, or maintenance of armed forces vessels. This definition includes "graywaters," weather-deck runoff, and ballast water. The statute also defines an MPCD as any equipment OR management practice, for installation or use onboard a vessel, designed to receive, retain, treat, control, or eliminate a discharge incidental to the normal operation of a vessel. UNDS is to be implemented gradually, through three phases (Figure 6). UNDS legislation requires consideration of seven factors in establishing regulatory requirements:

1. Nature of the discharge
2. Environmental effects of the discharge
3. Practicability of using an MPCD (device or practice)
4. Effect of MPCD on the operation of a vessel
5. Applicable U.S. law
6. Applicable international standards
7. Costs of MPCD installation and use

This presentation will provide an overview of the UNDS process through Phase I and Phase II.

In Phase I of the UNDS program, 39 liquid discharges incidental to normal operation of armed forces vessels were identified as potentially toxic to the environment. Twenty-five of these discharges were determined to require control by an MPCD. Fourteen discharges were determined to have a low likelihood of causing an environmental effect, and therefore, were determined to not require control. The final list of 25 discharges requiring control and of 14 discharges NOT requiring control was published in the Federal Register on 10 May 1999 and is accessible via the UNDS home page http://unds.bah.com/.
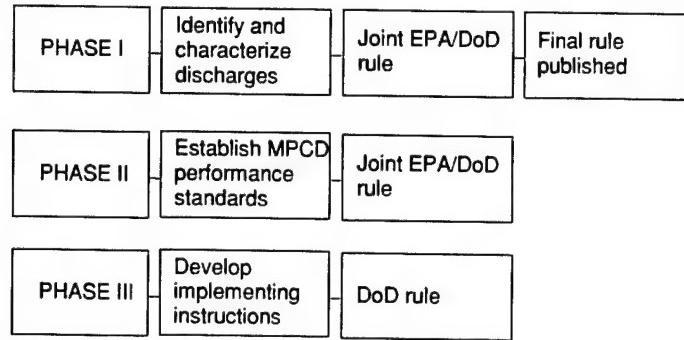
Figure 6. Phases and subphases of UNDS approach.

In Phase II of the program, which is currently underway, the U. S. EPA and DoD are developing performance standards for the 25 discharges that required control. Copper is a constituent in many of these discharges and may be addressed by some of the standards. The evaluation of performance standards initially requires identification and evaluation of potential MPCDs based on seven factors in the legislation. Standards are then based on the actual performance achieved by feasible MPCDs. Finally, target standards are established when existing MPCDs cannot achieve the desired performance.

Figure 7 provides a detailed diagram of the Phase II approach.
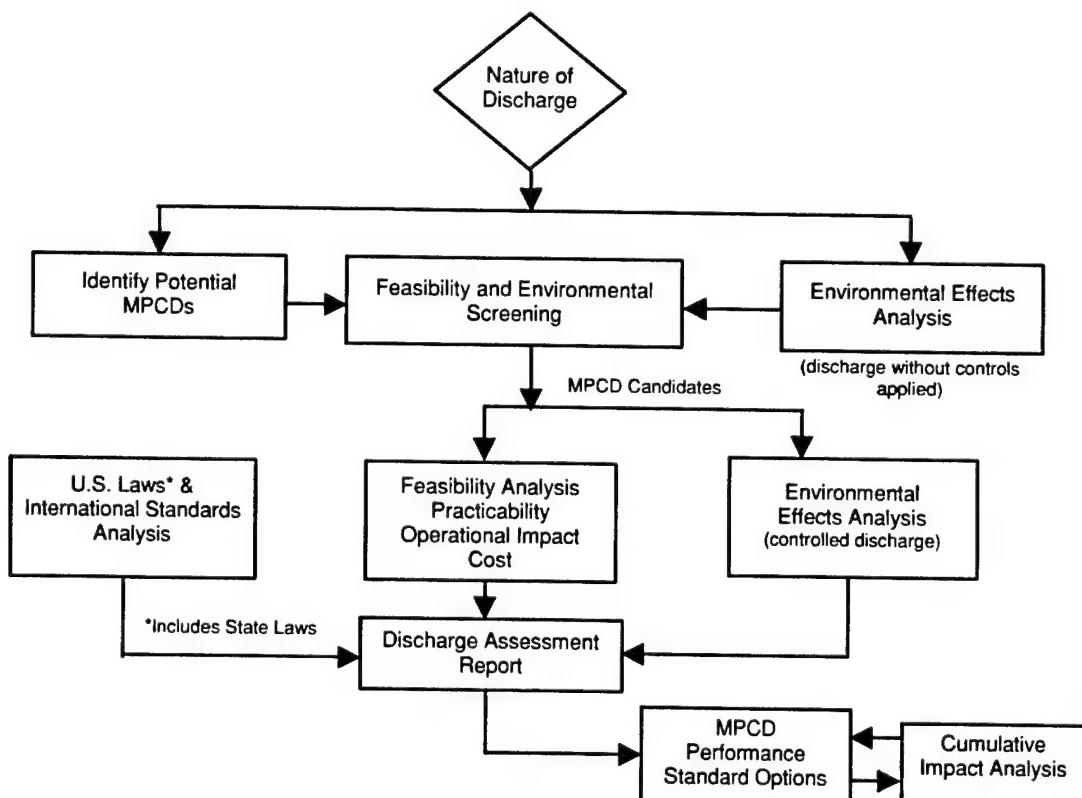
Figure 7. UNDS Phase II approach.

Potential sources of MPCDs are sought via announcements, Navy and Coast Guard technical reports, literature searches, and equipment experts. Some examples of the feasibility factors considered include space, volume, weight, overall safety, and suitability for application in the marine environment. Other factors, such as mission capabilities, personnel impact, etc., also apply.

Certain discharges have been identified as being potentially harmful to the environment, primarily through their copper content. These copper-laden ship discharges include

1. Hull coatings
2. Seawater cooling water
3. Firemains
4. Dirty drains
5. Hull husbandry
6. Submarine bilgewater
7. Steam condensate drain
8. Sonar dome
9. Compensated fuel ballast
10. Surface vessel bilgewater
11. Boiler blowdown

As an example of how the Phase II process works, for bilgewater, the program has identified nearly 60 individual MPCD options that can be divided into 12 technology categories or "option groups" including centrifugation, a gravity coalescer, a gravity coalescer + filter, and/or zero discharge in port with transfer to a shore-side facility. UNDS is currently conducting a feasibility and environmental effects analysis. Finally, the cost of procuring, installing, operating, and maintaining different MPCDs needs to be evaluated. The cost model includes the initial costs (acquisition, installation, and technical data development) and recurring costs (labor and waste disposal). The cost model needs to provide sufficient information to compare MPCD options.

ANTIFOULING COATINGS: CURRENT AND FUTURE (M. Ingle)

Introduction

This presentation summarizes the Navy antifouling coating needs from the perspective of technical and environmental requirements to maintain the Fleet in proper readiness and presents an overview of the current status of the Naval Sea Systems Command (NAVSEA) antifouling coatings program.

Underwater hull fouling increases drag and fuel consumption. All ships must, in some way, control this phenomenon, generally via the application of antifouling coatings (paints) and periodic, simultaneous removal of fouling organisms and portions of old paint during drydock maintenance, even if at great expense. At present, the Fleet and NAVSEA are reducing operating costs by extending the "drydock cycle" (the period between drydock maintenance) to 73 months. Presently, a 12-year antifouling coating life cycle is being projected (12 years between applications), and fleet maintenance activities (such as in-water hull cleaning) are extending this time effectively. The drive to extend the time between drydock periods and reduce operating costs sets the desired performance level of the antifouling coatings. The antifouling requirements of Navy vessels are usually more

demanding than those of commercial vessels because Navy vessels are kept at pierside longer. Historically, the preferred antifouling coatings were based on copper (a powerful algaecide) and tributyltin (TBT), a powerful biocide.

Unfortunately, better antifouling performance usually also means greater toxicity to the environment. While the need for more efficient antifouling coatings exists, the worldwide awareness of the need to protect our harbors and waterways is leading to more restrictive environmental regulations. For example, the International Maritime Organization (IMO) has drafted a binding document that would ban TBT coating applications in the year 2003 and eliminate all TBT coatings by 2008. Similarly, copper-based paints are undergoing extensive scrutiny. Local water-quality issues in ports with a large concentration of military and civilian ships such as San Diego Bay may one day restrict the use of copper-based paints. As part of the UNDS program, the Navy has agreed to investigate more environmentally acceptable coatings, such as epoxy or Teflon-like (low surface energy) coatings.

The environmental requirements are stringent, and future copper-based antifouling coatings will likely need to significantly reduce copper emissions to below 17 μg/cm^2/day (viz., laboratory measurements of panels alternately exposed to dynamic [MK+] and static conditions on a rotating drum release 17 μg/cm^2/day of copper). The NAVSEA goal is to reduce emissions to less than 10 μg/cm^2/day. Also, coatings must comply with current and future air emission regulations and must be applicable using "standard" personal protective equipment. Finally, coatings must be approved by the Navy Environmental Health Center (NEHC). This requirement presents a challenge for both the Navy and its commercial suppliers who must support the Navy with the investment required to obtain U.S. EPA registration on antifouling coating.

Directions of the Coating Program

The coating program follows essentially three technical options illustrated in Figure 8.

The first of these is to base the coating on a nonmetallic, nonpersistent biocide. This biocide may be incorporated in a "self-polishing" resin and must combine high toxicity with a short half-life of about 10 hours, degrading to harmless products. The biocide must control a broad spectrum of fouling organisms and support a 12-year drydock cycle without periodic hull cleanings.

The second option is to combine low copper emission with a short half-life biocide to prevent biofouling across a wide spectrum of organisms. This coating would reduce copper emissions to below the UNDS goal of < 10 μg/cm^2/day.

The third option is the environmentally most satisfactory if realizable. This option would develop low surface energy coatings, which would prevent the attachment of fouling organisms. The material would be silicone-based, completely nontoxic, and highly efficient in conserving fuel. A number of commercial "partners" are delivering prototype coatings in all three categories to the Navy for testing.

Testing and Risk Assessment

The NAVSEA Program Plan includes both analysis of environmental impact and assessment of coating performance. In this effort, NAVSEA has been working with SSC San Diego. The environmental analysis includes a hydrodynamic/contaminant transport model of copper "loading" (inputs, outputs, and steady-state concentrations) in San Diego Bay (Figure 9) and an assessment of coating test risk in accordance with 40-CFR-172.3 criteria. Coating performance evaluations include both laboratory tests and in-situ applications such as the placement of painted panels in seawater

environments and patch tests on Navy vessels. Table 1 gives the active ingredients employed by coatings in options 1 and 2.
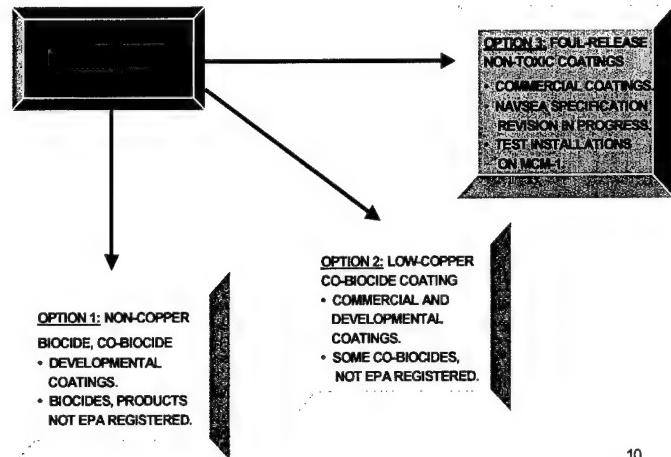
Figure 8. Options of the coating program.
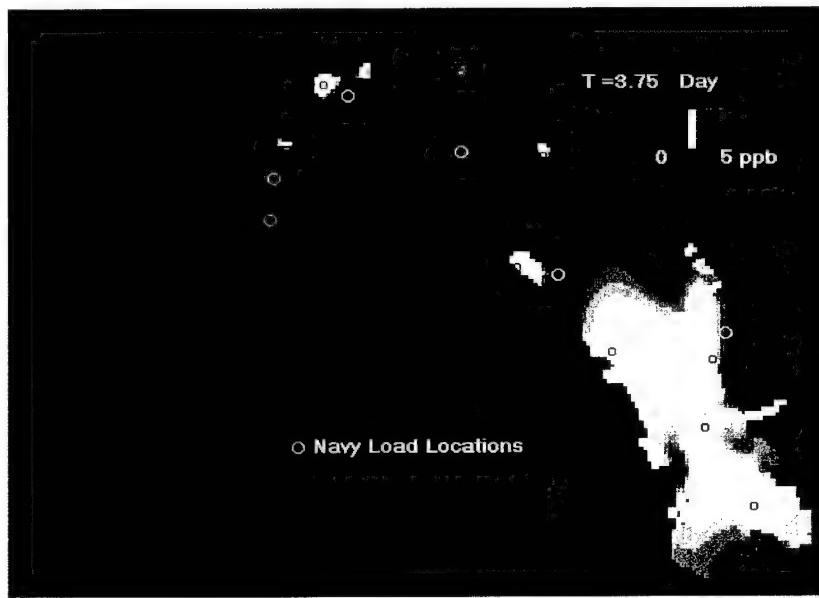
Figure 9. Simulated current Cu loading in San Diego Bay using the TRIM II hydrodynamic model.

23

Table 1. Active ingredients used in copper-based antifouling coatings.

COATING	USERS	ACTIVE INGREDIENT	HALF-LIFE OF ACTIVE INGREDIENT IN WATER
TRI-BUTYL-TIN (TBT)	OCEAN-GOING COMMERCIAL SHIPS;	TBT	~2-3 WEEKS (3.5 years Sediment)
	NOT USN SHIPS	Cu_2O	INFINITE
COPPER ABLATIVE	USN SHIPS COMMERCIAL RECREATIONAL	Cu_2O	INFINITE

OPTION 1	TBD	ORGANIC BIOCIDE	~3-15 HOURS
NON-PERSISTANT, NON-METALLIC		ORGANIC CO-BIOCIDE	~3-52 DAYS
		SEA-NINE 211	~ HOURS
OPTION 2	TBD	ZnPT	HOURS / INFINITE
LOW-COPPER, CO-BIOCIDE		CuPT	HOURS / INFINITE
		ZnO	INFINITE
		CuSCN	UNKNOWN / INFINITE
		SEA-NINE 211	~ HOURS
		Cu_2O	INFINITE

The current testing program includes the placement of newly painted panels exposed in semi-tropical and tropical waters where fouling tends to be rapid (San Diego Bay, CA; Biscayne Bay, FL; and Pearl Harbor, HI), and the placement of test patches on active Navy and Coast Guard vessels.

Summary

The Navy needs coatings with more effective, less persistent, less hazardous biocides to support increased drydock cycles. The environmental effects of these types of coatings will be best evaluated with ecological/community studies rather than by monitoring ambient concentration as is presently done. Foul-release (or nonstick) coatings offer unique benefits and are an important option for current and future Navy use. In the development of new coatings, NAVSEA would like to maintain an active dialogue with ONR and the research community.

NEW TECHNOLOGY FOR NEW ANTIFOULING COATINGS (E. Haslbeck and J. Montemarano)

Introduction

Advances in antifouling (AF) coating technology today are driven by environmental regulations and extended service requirements. It has been estimated that 75% of the research and development effort in the coating industry is focused on developing coatings that are "environmentally friendly" or comply with present or projected future environmental regulations. U.S. Navy ships have traditionally relied on copper antifouling paints for fouling control. There is a need for the Fleet to maintain operational readiness with practical and cost-effective antifouling coatings and dedicated hull husbandry.

Maintaining the Navy's ability to respond quickly to an operational requirement is possible when coatings perform well, cost little, require minimal maintenance, and are properly maintained. The Navy's primary problem with fouling accumulation is its significant contribution to hydrodynamic drag. Even microbial biofilms can increase drag by 10 to 20%. This problem leads to increased fuel consumption—an estimated $30M to $60M in fuel is wasted annually due to fouling-produced drag. This is a significant portion of the Fleet's approximately $500M annual total propulsive fuel bill.

The Navy places unique demands on its antifouling coatings. As opposed to the commercial shipping fleet, Navy vessels spend long periods of time at pierside, where the majority of fouling occurs. Drydock intervals have traditionally been 5 to 7 years and are now being extended to 10 to 12 years. This means that coating-system-performance life is being pushed to the limit; the period between blasting and repainting is being extended. The Navy's ultimate goal is a paint requiring a drydock interval greater than 20 years (Figure 10). If the current copper ablative AF coatings were used under these operational life cycles, it would be necessary to conduct more underwater hull cleanings and more in-situ hull/coating repairs.

New polymer or resin systems and more restrictive legislation on air and water quality at the local, national, and global level have led to the development of several emerging coating technologies. Focus on the negative effects of biocides has increased due in part to the ban on TBT-based coatings by the IMO. Accordingly, the environmental effects of copper released by antifouling coatings are coming under increasing scrutiny. Within the U.S., the UNDS effort, a partnership between the U.S. EPA, DoD, and the states, has identified copper as a discharge of concern from hull coatings and from hull-cleaning operations. In Canada and Sweden, restrictions on the use of copper have been implemented. Restrictions within the U.S., perhaps limited to particularly affected regions, are anticipated.

Figure 10. U.S. Navy ship in drydock for hull servicing (top) and representative marine fouling (bottom).

To be successful, new coatings for fouling control must maintain the required operational service life while simultaneously meeting restrictive environmental standards. To do so, well-defined analytical techniques are needed that accurately characterize the performance of antifouling coatings under a number of environmental conditions. The same techniques would also permit the evaluation of the coatings' environmental impact.

AF Coatings Under Development

For quite some time, it has been known that tons of copper are annually released into harbors, drydocks, and waterways. The effects of this are still not completely understood. Therefore, over the past 20 years, considerable effort has been put into the development, testing, and evaluation of low toxicity or nontoxic coating technology. Copper ablative AF coatings have been in use in the Navy from the 1980s. Generally, such coatings are rosin-filled, acrylic resin systems that contain 40 to 55% cuprous oxide and 20% zinc. Recently, many paint companies have experimented with, and eventually marketed, versions containing organic booster biocides or co-biocides. Nontoxic silicone, easy-release coatings are designed to minimize the strength of adhesion between the fouling organism and the slick silicone surface. In theory, the coating may allow fouling to settle, but under flow (e.g., motion of ship through the water), the fouling will release (i.e., the hull will self-clean). Silicone coatings contain no biocides and are effective through a nontoxic mechanism.

At the same time, research in the area of "environmentally friendly biocides" has led to the development of new organic "booster" biocides. It may be possible to reduce the amount of copper needed to control fouling through the addition of co-biocides. The new co-biocides have been designed and selected to biodegrade rapidly in the marine environment—therefore minimizing concerns over bioaccumulation and persistence. Several co-biocides have been identified, and a combination of
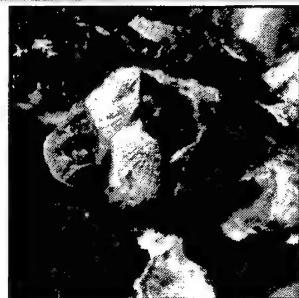
25

these co-biocides may prove to be effective against a relatively broad range of fouling organisms. Such co-biocides could offer the potential to reduce even further, or even possibly eliminate, the need for a metal-based biocide such as copper.

Self-polishing polymers have existed for several decades, and were originally designed to control the release and polishing rate of organotin-based coating systems. Over the past several years, however, this concept has been adapted and engineered to control the release and polishing rate of tin-free copper-based AF coating systems. Virtually all copper self-polishing coatings are used in combination with co-biocide technology to achieve the desired AF performance.

Adoption of New Coatings

Any changeover in coating technology raises a number of questions about the new material's suitability and ability to meet the end-user's needs. Generally, the main criteria for judging a coating are (1) its effectiveness in controlling fouling and (2) its potential to meet environmental standards. If the coating is not environmentally compliant, it will not be marketable, and if it does not effectively control fouling, it will not be particularly useful. Additional hurdles must be overcome before the new technology is introduced. These hurdles include ease of application and removal, adhesive qualities, ability to overcoat, material cost, maintenance requirements, performance under flow, wear rate, and anticipated performance life. A new coating technology, in general, is desirable only if it either equals or outperforms the previous state-of-the-art on many of these levels.

Conclusion/Future Directions

The multiple demands currently being placed on copper-based AF coatings, increased effectiveness, longevity, and reduced toxicity call for new developments in AF-coating technology. To adequately and comprehensively assess the performance and potential of a new coating type or class of coatings, the proper tools and performance characterization criteria must be identified and implemented. A more comprehensive evaluation of the underwater hull-coating system has been made possible by using a free-swimming, remotely operated vehicle (ROV) (Figure 11). Newly developed sensors on the ROV measure paint thickness underwater and document the coating condition in terms of fouling resistance and coating integrity by using digital video cameras.

Figure 11. U.S. Navy ROV.

Automated underwater vehicles offer the promise of providing not only in-water hull evaluation but also in-water maintenance by using environmentally compliant hull-cleaning techniques. (See Bohlander; McCue papers in this report.)

Development of new coating-assessment tools and procedures is essential for the effective characterization of existing and emerging coating technologies. Recent studies emphasize that measuring and understanding biocide release rate (by using both laboratory and in-service practices) is not straightforward. Each release-rate measurement technique has its advantages and limitations. Field techniques are environment-specific in that the results are tied to the make-up and presence or absence of biofilm, and parameters such as temperature, pH, and salinity. Laboratory techniques (Figure 12) set up arbitrary conditions of hydrodynamic flow, pH, salinity, and temperature and do not accurately reflect realistic in-situ environmental loading. This point has been raised in the literature and remains an important issue where environmental loading calculations are concerned. Estimating environmental loading and impacts from release-rate data is also difficult as Cu speciation and complexation must be taken into account.
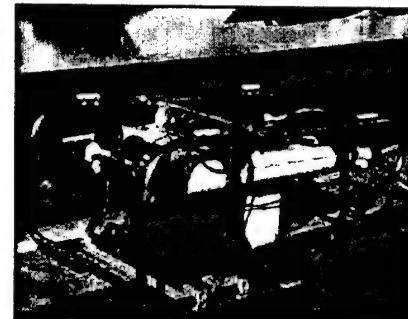
The ability to determine the suitability of a new AF material requires that the appropriate protocols, practices, and techniques be properly understood and engineered to produce the data and results needed to make a responsible decision. New and innovative techniques are needed to accurately quantify the coating performance, the biocide emission, and the environmental impact of the new coatings.
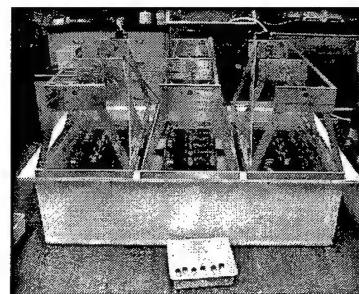
Figure 12. Laboratory AF paint leachate experiment.

IN-WATER HULL CLEANING: COPPER SOURCES AND SOLUTIONS (G. Bohlander)

The goal of our program is to develop and demonstrate an underwater hull-cleaning system to remove marine fouling from Navy hulls while capturing and containing effluent. The effluent contains cuprous oxide, zinc oxide, marine fouling organisms, and paint particles and is considered harmful to the environment. The approach is to develop an advanced hull maintenance vehicle (AHMV) and to demonstrate and integrate technologies for

1. cleaning tools for both copper and foul release paints
2. effluent management and processing
3. a remotely operated vehicle with automated navigation and operation
4. acoustic imaging, not affected by turbidity, and
5. special maintenance sensors.

Unlike the Submerged Cleaning and Maintenance Platform (SCAMP), which was operated by divers and had no effluent collection system, the AHMV is an autonomous, hull-adhering vehicle, which can be either diver-operated or remotely controlled to efficiently remove fouling from the AF paint system, while simultaneously collecting the brush effluent for safe disposition at pierside. Figure 13 illustrates this concept.
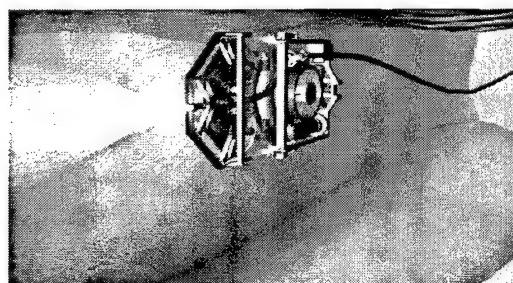
Figure 13. Artist's concept of the AHMV.

The second prototype of this vehicle, named RM2 for Research Model 2, was completed in early 1999. An evaluation of the system was conducted in David Taylor Model Basin in February 1999, and the first ship deployment was conducted on USS *Cape St George* (CG 71) in April 1999.

Modifications included a new deck-forward design, which was more efficient in both hull cleaning and capturing the effluent, and improved maneuverability and control. Figure 14 shows the RM2 after successfully cleaning *Cape St. George* and shows the cleaning brushes in the new deck-forward design.
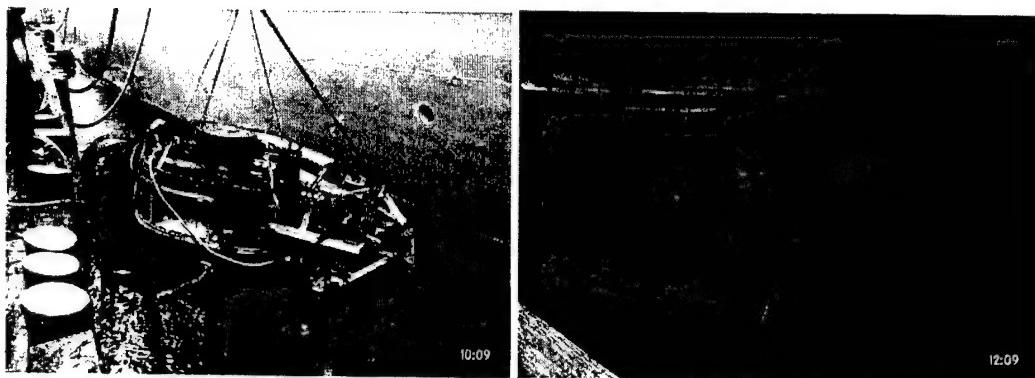
Figure 14. View of the AHMV at work (left) and underside of hull-cleaning brushes (right).

The AHMV was judged to be roughly equivalent in cleaning effectiveness to its precursor, SCAMP, but removed only half of the paint per cleaning, a distinct advantage. Figure 15 indicates the performance of the AHMV.
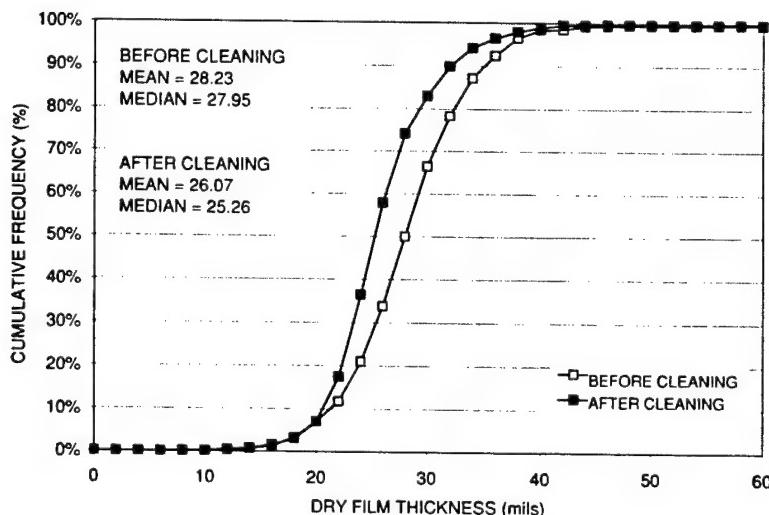
Figure 15. Dry film thickness (DFT) on USS *Cape St. George* before and after cleaning with the AHMV.

The RM2 model with a centered and forward articulated deck and improved seals also successfully captured the cleaning effluent. The effluent was transported via flexible tubing 450 ft to a pier for discharging. A preliminary design for an effluent pre-filter was evaluated to study its potential for reduction of copper particulates. It was found that the pre-filter lowers the Cu content of the effluent by approximately 50%, leaving a Cu content that ranges from 1 to 30 ppm. The volume of effluent ranges from 50K to 350K gal depending on ship class.

A preliminary evaluation of copper removal technologies was conducted, and three methodologies were investigated: ion exchange resins, immobilized ligands, and polymeric-coated activated carbon. All were found to be effective. Ion exchange resin lowered the Cu content from 0.3 ppm to 9 ppb, while ligands effectively lowered the copper content from 6.6 ppm to 18 ppb, and coated carbon successfully dropped it from 11.6 ppm to 9 ppb. Note that these tests were conducted individually and

that the Cu and Zn concentrations in the effluent stream varied depending on paint condition and fouling accretion. Also, these were bench-scale tests, which did not consider scale-up issues at the time.

Underwater hull cleaning is an UNDS discharge, and the UNDS process may influence regulatory discharge limits. Future plans call for continual improvements to AHMV in both the hull-cleaning system and in the development of an effluent treatment system. A next-generation "prototype" vehicle is to be built under the management of the Supervisor of Diving and Salvage at NAVSEA, and further effluent treatment evaluations are to be conducted.

FUTURE UNDERWATER HULL-CLEANING TECHNOLOGIES (T. McCue)

As mentioned in the previous articles, the operational readiness of U.S. Navy vessels is paramount, and the ability of ships to successfully perform their mission is directly related to the routine maintenance conducted on the underwater portions of these ships. One critical aspect of this maintenance is underwater hull cleaning. This procedure removes fouling organisms and renews the effectiveness of AF coatings, without placement of the ship in a drydock for an extensive (and expensive) period during which normal ship's operations are precluded. Therefore, to sustain the readiness of the Fleet, it is important that we pursue advancements in underwater hull-cleaning technology and simultaneously improve the cleaning processes to mitigate its impact on the environment.

The Navy's shipboard-waste-management RDT&E program is currently developing "mature" underwater hull-cleaning and effluent treatment technology. Concept exploration of treatment technology began in FY 2000 with a scheduled demonstration and a validation scheduled in FY 2003. A final implementation of an integrated full-scale prototype cleaning and treatment system is scheduled for 2005.

Current, in-situ, hull-cleaning technology consists of an integrated, modular system design that features a mobile vehicle capable of both cleaning and treating captured effluent (see Bohlander, previous article). The actual cleaning subsystem, attached on an umbilical cable, is diver-operated and may be supervised from the pier (or any other location) by using the video cameras installed on the cleaning vehicle. The cleaning is easily performed pierside, thereby having a minimal impact on ship's operations (Figure 16).
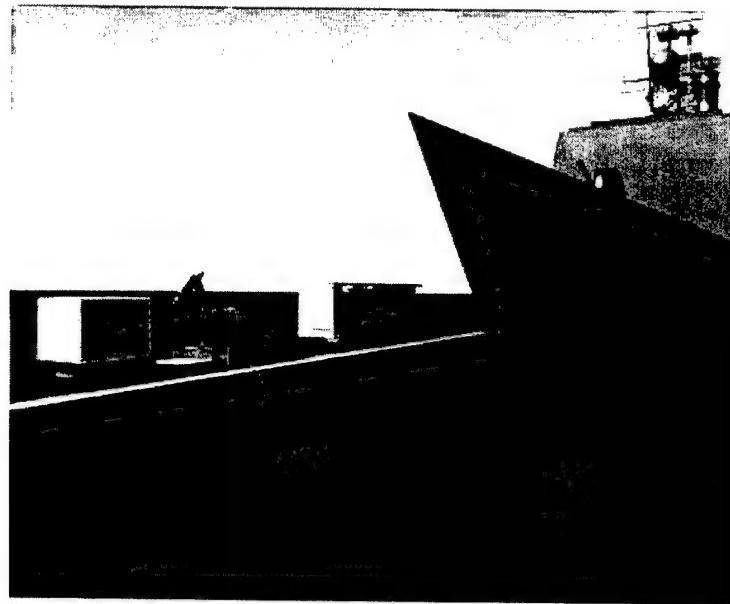
Figure 16. Artist's rendition of the underwater hull-cleaning system and mobile support modules.

The prototype cleaning vehicle has an overall size of 108" x 76" x 29" and cuts a 58-inch swath with center axis turning. The vehicle transports both sonar and navigation systems via an Ethernet transmission to the topside supervisory console. The vehicle is also capable of transporting maintenance sensors used to measure the hull electro-potential, hull-coating thickness, and hull-plate thickness. Figure 17 displays the device.
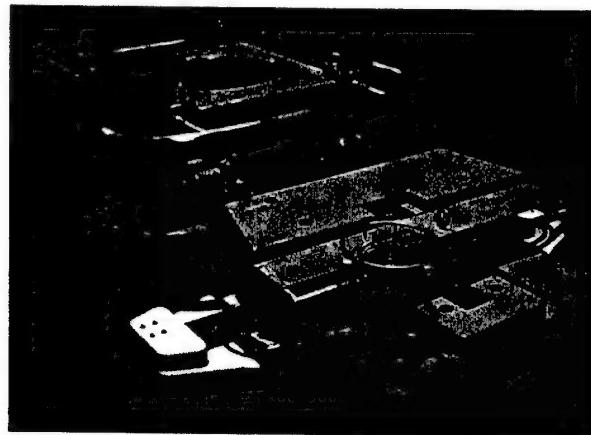
Figure 17. Diver-operated cleaning subsystem.

During hull-cleaning operations, the system generates an effluent containing a dissolved and particulate phase. The dissolved portion consists mostly of copper and zinc from the brushing action on the coating. The particulate phase contains abraded AF coating: copper and zinc; partial or entire calcareous marine organisms, including barnacles, tube worms, oysters, and coral. Also, there are filaments of hydroids, grasses, algal mats, slime, and, of course, microorganisms.

The discharge from one hull-cleaning unit and two smaller hand-held units is 500 GPM. This large volume of material must be processed "online" before it can be disposed of, and the system must be able to process discharge continuously for the 10-hour period of cleaning. It is essential to remove particulates and mitigate discharge of heavy metals to meet regulations. The processed effluent may then be returned to the surrounding waters. Particulates may be stored in holding tanks and packaged for transport and disposal or recycling. Many potential effluent treatment technologies are being considered at this time (Table 2):

Table 2. Possible effluent treatment technologies.

Recommend for Bench-scale Testing
Sand-ballasted Coagulation-Sedimentation, Crossflow Tubular Membrane Separation, Particulate Reagent
Electro-coagulation JOUSE ECTM System
Absorption using Chemically Surface Modified Gel (GSMG)
Dynamic Phase Separator Filter Device (Sintered Metal-Screen Discs)
Secondary Recommendations for Bench-scale Testing
Absorption using Enviro-Clean Process
Dissolved Air Floatation, Mechanical/Mixed Media Filtration, Absorption
Mechanical Separation, Absorption, Precipitation, and Clarification
NOT Recommended for Bench-scale Testing
Modified Filtration and Sorption (Rotoshear Screen, Carbon-Coated Biofoam Filter (CCBF) /Triad Moving Belt Contactor)
Precipitation of Cu and Zn Sulfides with Secondary Micro Filtration
Multi-Stage Mechanical Screen, Bag Filter, Carbon Absorption, Two-State Membrane (MF(ZEEWEEDO) and UF) with Reject Stream Drying
Mechanical Separation, Filtration, Electro-coagulation and Clarification with Alternative Absorption
Mechanical Separation, Precipitation, Biological and Proprietary Filtration
Prefilter, Absorption, and Mechanical Press

The planned stages of development of the in-water hull-cleaning system include

1. the fabrication of a prototype cleaning vehicle

2. a feasibility study of treatment systems

3. a cost evaluation of the treatment systems

4. a prototype development of the system

5. a full-scale development of the treatment system, including the integration of the cleaning system

6. a full-scale test and evaluation of the cleaning and treatment system

7. the establishment of performance specifications and transition to the field

The end result of the project will yield an advanced hull-cleaning system capable of mitigating the discharges generated by the cleaning process. Such an advance will broaden the Navy's hull-cleaning capabilities and help to sustain the operational readiness of U.S. Navy vessels.

COPPER SOURCES AND LOADING IN NAVY HARBORS (P. F. Seligman, H. D. Johnson, A. O. Valkirs and J. G. Grovhoug)

The objectives of this short-term study were to evaluate copper loading in Navy harbors from both Navy and non-Navy sources. Evaluated harbors included San Diego, CA; Pearl Harbor, HI; Norfolk/Little Creek, VA; Bremerton WA; and Mayport, FL.

Methods

Site visits were made to each harbor to collect data on site-specific conditions, vessel counts, and other related parameters. A literature survey was also conducted for all locations, and data gaps were defined. An estimate of copper-loading from watershed, nonpoint sources was calculated from a Geographic Information System (GIS) analysis of United States Geological Survey (USGS)-defined watersheds and existing reports on stormwater monitoring. Existing Navy measurement data were collected from hull (both Navy and pleasure craft) AF-coating copper-release rates, vessel discharges, in-water hull-cleaning discharges, and NPDES permit measurements, all of which were used as components of the loading assessment (Johnson et al., 1998). A unique Navy in-situ dome system that attaches to the side of vessels by using light suction and that collects water samples over time was used to measure Cu release rates from hull AF coatings of Navy ships and pleasure craft under environmentally realistic conditions (Lieberman et al., 1985; Seligman et al., 2001). These measurements yield lower values than traditional laboratory methods but are considered to be a better estimate of environmental loading because they are made under natural conditions (Figure 18). These conditions include an intact biofilm and ambient water conditions where pH, salinity, and temperature may affect release rates as well. Other loading factors such as air deposition or unmeasured stormwater were derived from model estimates.
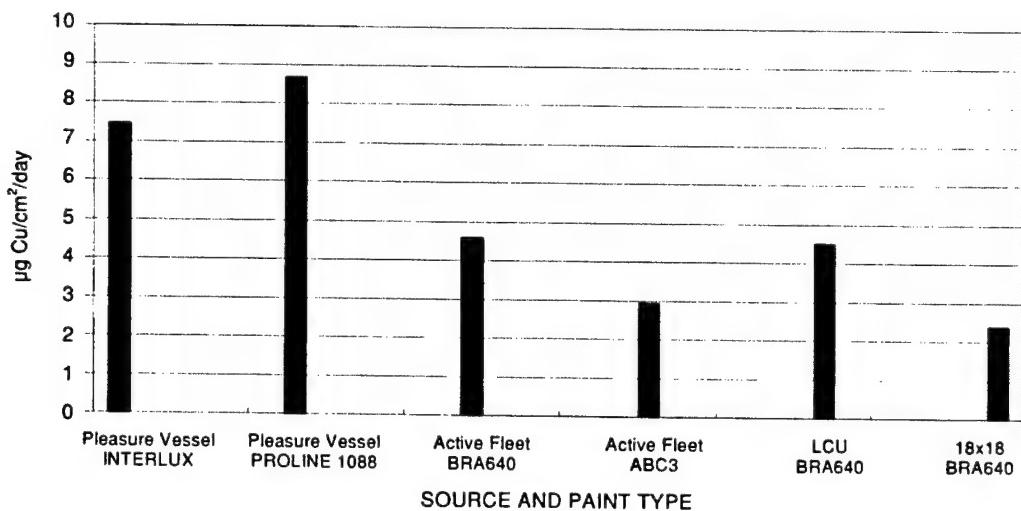
Figure 18. Mean in-situ release rates for pleasure craft, active Navy vessels, and stationary 18- by 18-inch panels.

Loading Estimates

Stationary 18- by 18-inch panels, typically with heavy layers of biofilm, had the lowest Cu release rate values, approximately 2 $\mu g/cm^2$/day, while Navy vessels averaged 3.9 $\mu g/cm^2$/day, and pleasure craft had twice that value or approximately 8 $\mu g/cm^2$/day. Additionally, a single Navy Landing Craft Utility (LCU) water craft, which was used as a test vessel for application of several AF test coatings,

had an average release rate of approximately 4.5 $\mu g/cm^2/day$, similar to the same paint system on the six larger vessels for which rates were measured.

These in-situ hull release rates are used in conjunction with other measurements and estimates to develop a total dissolved Cu loading estimate for each Navy harbor. Figure 19 provides total dissolved Cu loading by harbor, including Navy and civilian loading from hull-coating leachates. This figure has been modified to include new in-situ release-rate data taken since the November 2000 workshop.
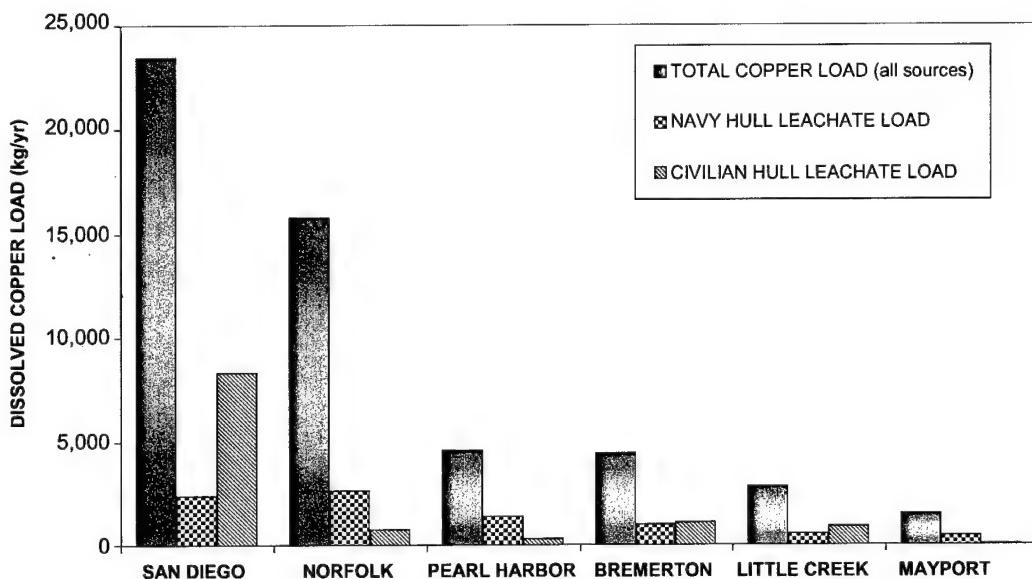
Figure 19. Copper loading for each Navy Harbor calculated from measurements, published sources, and estimated values.

As seen in Figure 19, San Diego and Norfolk have the highest copper load, 23,000 Kg and 16,000 Kg, respectively. The higher Cu load in San Diego Bay is attributed to the relatively large number of private craft (over 8,000) that reside within the Bay. In addition, loading from small craft is elevated relative to Navy hull leachate because of higher civilian release-rate values used in the loading calculation (twice that measured for naval vessels). Private craft in San Diego are painted more frequently with typically one of two commercial formulas of AF paint and are cleaned on a more regular (generally monthly) basis. Frequent hull cleaning may also reduce biofilm accumulation on private-craft hull surfaces and cause an increase in release rate.

Summarizing the loading data in regard to hull leachate, Cu loading from civilian hull AF-coating release exceeds Navy hull-coating release in San Diego and Little Creek, whereas loading from Navy hull-leachate exceeds civilian hull-leachate loading in Norfolk, Pearl Harbor, and Mayport. Civilian and Navy hull-leachate Cu load are nearly the same in Bremerton.

Other significant Cu loading factors include ship discharges such as cooling water, civilian-craft hull cleaning, stormwater runoff, and shore discharges from permitted facilities such as shipyards and sewage treatment plants. In-water hull cleaning of naval vessels represents 1% or less of the total copper load to Navy harbors. However, in-water hull cleaning can produce short-duration plumes with high concentration of particulate Cu that can contribute to sediment loading and future regulatory or cleanup problems. Estimated loading from civilian hull cleaning ranges from essentially

0% in Bremerton to 23% of the total load in San Diego Bay. In San Diego, and likely at other warm-water locations, boat owners typically have their hulls cleaned on a monthly basis. This process can contribute substantial copper load when the AF-paint surface layers and biofilm are removed.

Water-Column Concentrations

Copper loading to urban harbors ultimately results in a steady-state Cu concentration in the water column. Figure 20 shows water-column concentrations of Cu in San Diego Bay measured in 1997. Due to loading and flushing patterns, the south-central portion of the bay, including the Naval Station, showed levels that were near or exceeded the chronic water-quality standard for California of 3.1 µg/L. The interior of yacht harbors often exceeded the Cu water-quality standard because of low flushing rates combined with high loading from private yachts.
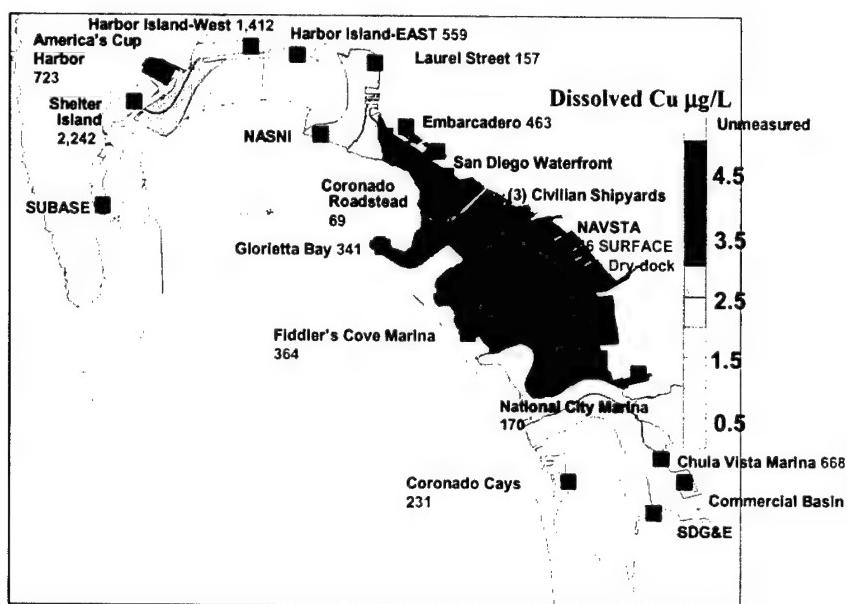
Figure 20. Copper concentrations (ppb) and sources in San Diego Bay (Katz, 1998). Numbers represent number of vessels in yacht harbors/Navy areas.

Conclusions

- Copper from antifouling coating leachates from both Navy and private vessels constitutes a significant portion of the total Cu loading into Navy harbors.

- Other major Cu sources include nonpoint inputs (stormwater), hull cleaning of private vessels, ship discharges such as cooling water, and shore-point sources such as shipyards.

- Loading sources and total Cu loading vary widely between harbors by as much as a factor of 10 (e.g., between San Diego and Mayport).

- In-water hull cleaning of Navy vessels typically represents less than 1% of the total loading. Civilian-craft hull cleaning, however, can add substantial Cu loads to harbors where there are many small craft.

SCIENCE GROUP PRESENTATIONS

WATER-QUALITY CRITERIA / CU CONCENTRATIONS /CU ACTIVITY

(H. E. Allen, D. Ditoro, P. Paquin, and R. Santore)

The determination of copper in effluents and in estuarine receiving waters to comply with the U.S. EPA WQC presents two main difficulties: (1) the determinations are very difficult to carry out without contamination during sampling and analysis, and (2) total concentration itself is not a reliable indicator of actual copper toxicity. The first problem is solvable through the use of so-called "trace-metal clean" techniques pioneered by K. Bruland and co-workers. These techniques call for extraordinary caution in sampling (all equipment must be plastic and acid-cleaned) and during analysis (all sample handling and transference must take place in clean rooms equipped with class 100 hoods). Figure 21 provides an example of how sets of trace-metal measurements collected from New York harbor by "routine" techniques may differ from measurements collected by "clean" techniques.
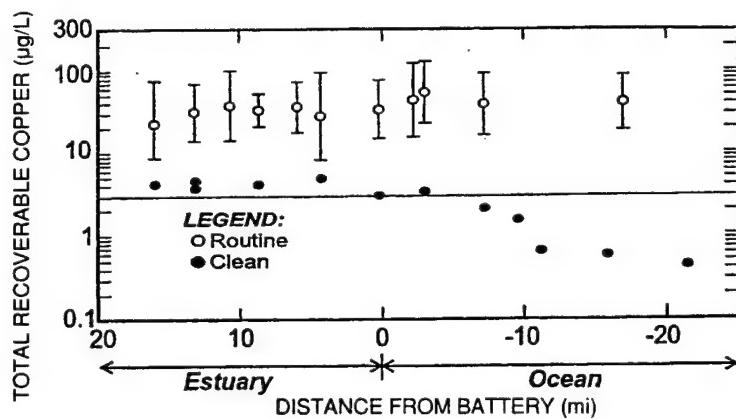
Figure 21. Copper measurements in New York Harbor.

Figure 21 shows that routine analysis would lead to the conclusion that the WQC have been grossly exceeded, while the values are, in fact, very close to the present WQC of 2.9 µg Cu/l (dissolved). The environmental "picture" is also clouded by the fact that many chemical forms or species of copper measured in "total recoverable" copper may, in fact, be present in nonlabile forms or species, and may not be toxic.

The speciation problem is presently addressed via several approaches to the use of WQC to develop site-specific standards for protection of aquatic life. The first approach is to use total recoverable metals analysis for surface waters and compare results to the criteria. If the values are below the WQC, then there is no problem, and there is no point in making further measurements. However, total recoverable metal is an essential parameter for waste-load calculations. When total recoverable metals exceed the WQC, then dissolved metals (the fraction that passes through a 0.45-µm filter) may be measured. This fraction is closer to the labile or toxic component, since much of the metal may be present in particulate or nonlabile forms. The third approach involves the determination of the WER. This procedure is a mechanism for the development of site-specific criteria. It is a means of indirectly addressing questions of metal speciation, and it makes a provision for the presence of metal-binding (viz., detoxifying) natural components in the receiving water. (National criteria are based on bioassays in laboratory water). Toxicity tests are performed with at least two species in both

local receiving water and laboratory toxicity testing water. The WER is the acute (or chronic) value in site water divided by the acute (or chronic) value in laboratory water. This ratio may be used to adjust the national or slate criterion to a site-specific value.

Bioavailability and Toxicity

The question of the bioavailability of copper and, therefore, its toxicity, is directly related to its physico-chemical form in the aquatic medium via its speciation. Physically, copper in water may be thought of as being present in a continuum of forms of increasingly smaller dimensions, from particulate, to colloid, to dissolved complex, to the hydrated ion (Mackey and Zirino, 1994). The definitions of the species are method-dependent, namely, the quantity in a particular fraction depends on the technique used. Particles and colloids may be obtained by filtration (0.45-μm filter and ultrafiltration); on the other hand, the truly dissolved species are much more difficult to separate. Dissolved complexes of copper may run the gamut from copper tied to humic-acid type macromolecules to copper bound to small molecular-weight organic molecules (e.g., amino acids), to purely inorganic moieties such as $CuCl_2^{\circ}$. Voltammetry is a technique that is routinely used to differentiate between labile and inert species. This differentiation is based on thermodynamic stability and on diffusion constants (and, therefore, also on molecular weights). Only potentiometry can differ among the inorganic species of copper (Belli and Zirino, 1993). However, the percentages of the individual species may also be calculated from an ion interaction model, if the total concentrations of components and the individual species' formation constants are known (Zirino and Yamamoto, 1972). Bioavailability is tied to lability; thus, the most reactive or available forms of copper are deemed to be bioavailable and therefore most toxic. The hydrated Cu ion is deemed the most labile. This factor has lead to the free ion activity model (FIAM) of Cu toxicity, which is well supported by the experimental data (Campbell, 1995). Zinc data also appear to support this type of model.

The Biotic Ligand Model (BLM)

The aquatic BLM combines the gill surface interaction model, originally proposed by Pagenkopf et al. (1974), and a metal ion interaction model to relate Cu speciation directly to toxicity (DiToro et al., 2001). To date, this model has only been applied to toxicity in freshwater; however, there is no a priori reason preventing its application to estuarine waters. Essentially, Cu ion toxicity to larval fathead minnow can be related to copper concentration in solution and to the corresponding amount of copper retained on the minnow's gill surface. Copper concentration in solution can also be converted to copper activity (taken to be the concentration of the hydrated ion in this discussion) by using a complexation model that contains both inorganic species and humic and fulvic acid-type compounds. Thus, copper activity is related directly to toxicity (Figure 22). A further refinement made by DiToro et al. (2001) is that the copper retained on the gill is simulated as a complexation on a hypothetical ligand where a competitive equilibrium exists between copper ion, sodium ion, calcium ion, and free protons (Table 3).
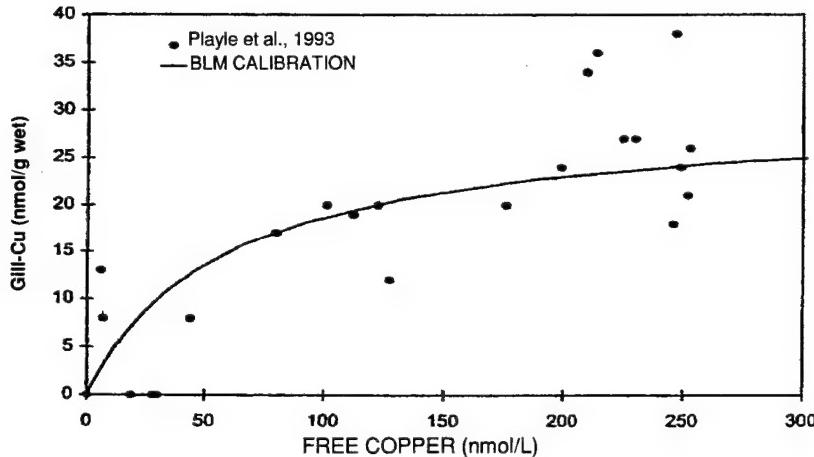
Figure 22. Measured copper accumulation on fathead minnow gills and biotic ligand model predictions as a function of cupric ion concentrations (Santore et al. 2001).

Table 3. Stoichiometry and thermodynamic formation constants for uptake of metals and protons on gills of larval fathead minnows (after Santore et al., 2001).

Reaction			Log K
H^+ + gill	$\longrightarrow$	H-gill	5.4
Na^+ + gill	$\longrightarrow$	Na-gill	3.0
Ca^{++} + gill	$\longrightarrow$	Ca-gill	3.6
Cu^{++} + gill	$\longrightarrow$	Cu-gill	7.4

Thus, the various factors that mitigate toxicity, such as quantity of organic matter, water hardness, pH, and sodium and calcium ion activity can be incorporated into the model to yield realistic results. Figure 23 is a schematic diagram of the BLM.
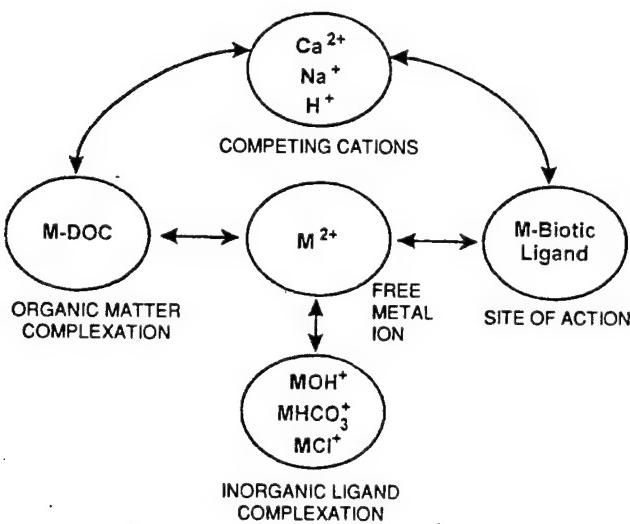
Figure 23. Conceptual diagram of the BLM showing inorganic and organic complexation in the water and interaction of metals and cations on the biotic ligand. DOC = dissolved organic carbon.

For fathead minnow larvae in static 96 h copper toxicity tests, the predicted results obtained with the BLM compared very well against the experimentally obtained LC50 values (Figure 24).
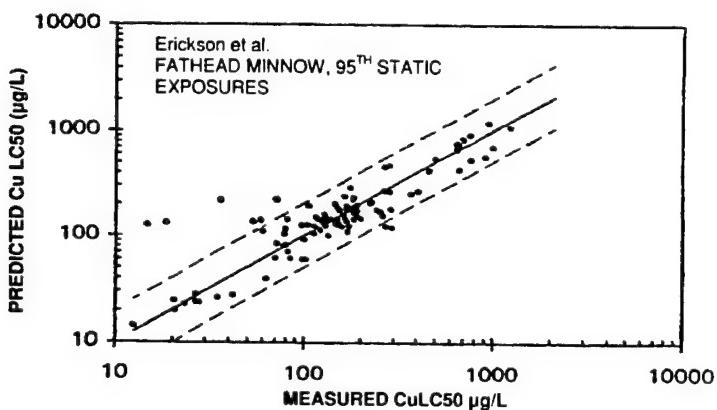
Figure 24. BLM predicted vs. experimentally obtained LC50 values for fathead minnows in static exposure tests (Santore et al., 2001)

In addition, predictions compared well with the LC50 values obtained in WER studies, also with fathead minnows. When the BLM was applied to the fairy shrimp, *Daphnia pulex*, the comparison between predicted and experimental values was also encouraging, suggesting a wider future application for the BLM in freshwater. However, more data are needed before the BLM can be applied to estuarine waters.

38

FREE COPPER ION ACTIVITY, COMPLEXATION CAPACITY AND TOXICITY IN SAN DIEGO BAY WATERS (I. Rivera–Duarte, G. Rosen, D. Lapota, and A. Zirino)

For the past two decades, the concentrations of total copper in San Diego Bay have remained relatively constant in the range of 0.9 to 3 µg L^{-1} (14 to 47 nM). The latter is the U.S. EPA's salt-water WQC, developed from toxicity studies on larvae of *Mytilus sp*. The indication is that some waters in San Diego Bay are unsafe for this species. However, these studies are based on total copper concentration, instead of free copper ion activity, which, according to the free ion activity model, is probably the best single indicator of the toxicity of copper to organisms. Following the works of Zirino, De Marco, Mackey, and colleagues (see paper by De Marco, this report), which indicate that the copper ion-selective electrode (Cu-ISE) provides a measure of the activity of the free copper ion (pCu) directly in seawater, we used it to study the relationship between pCu and the water quality of San Diego Bay. Direct measurements of San Diego Bay samples with the Cu-ISE yielded a range of pCu values between 11.3 to 12.6, with an average (µ) and standard deviation (σ) of 11.9 and ± 0.4, respectively. These values were consistent with those obtained for coastal and estuarine waters by other investigators who calculated pCu values from indirect (principally voltammetric) measurements: range 10.9 to 14.1; µ=12.1, σ ±1.0, (Figure 25).
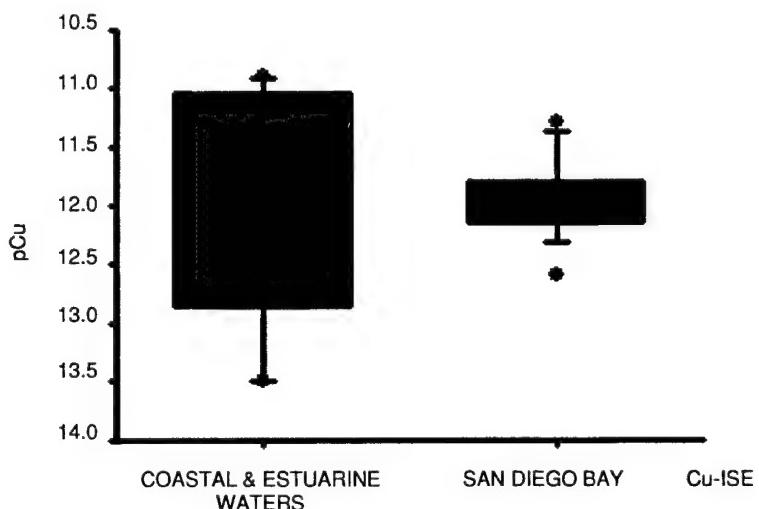
Figure 25. Comparison of pCu values directly measured in San Diego Bay with literature values of samples from estuarine and coastal locations. The line within the boxes is the median, and the boundary of the boxes indicates 25th and 75th percentiles. Error bars to the top and bottom of the boxes indicate the 90th and 10th percentiles. Closed circles show outlying points.

Direct measurements of copper complexation capacity (CuCC) with the Cu-ISE in unfiltered samples from San Diego Bay produced a range of concentrations (2.0×10^{-8} to 2.8×10^{-7} moles of Cu equivalents) also comparable to those (L_1 plus L_2; 1.1×10^{-8} to 5.7×10^{-8} moles of Cu equivalents) previously reported (Figure 26).
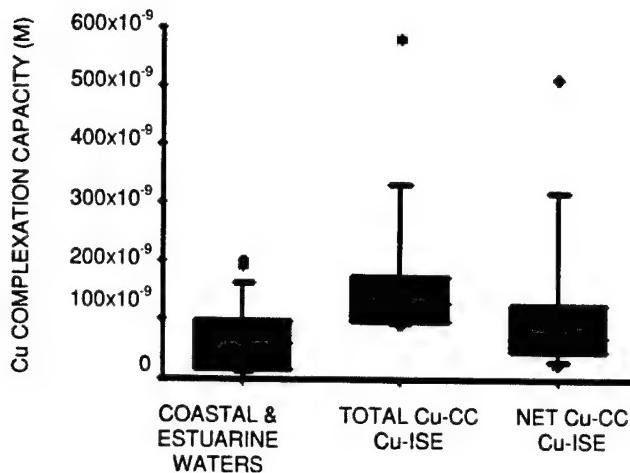
Figure 26. Comparison of CuCC values directly meas-
ured in San Diego Bay with literature values of samples
from estuarine and coastal locations. Description of boxes
is given in Figure 25.

To determine the role of CuCC on the water quality of the bay, the measurements with the Cu-ISE
were complemented with static toxicity tests on larvae of *Mytilus galloprovincialis*, following proce-
dures accepted by the U.S. EPA. These tests indicated a direct correspondence between the EC_{50} and
Cu-CC measured with the ISE (Figure 27).
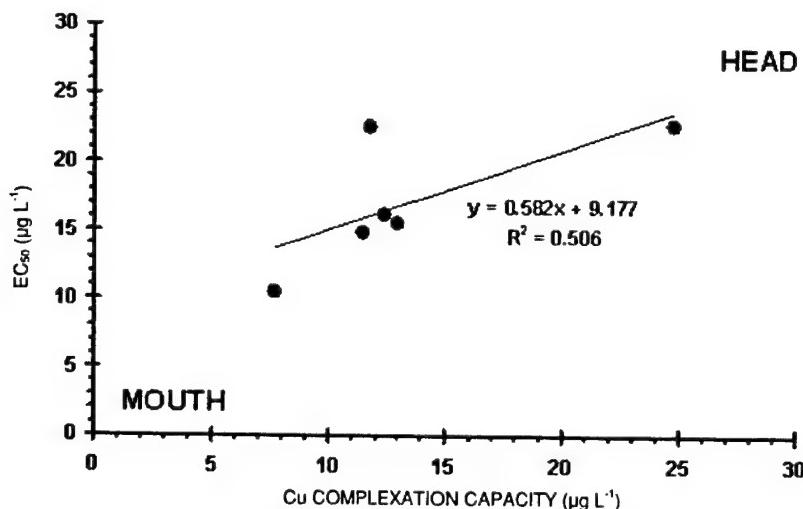
Figure 27. Relationship between bioassay results: CuCC measured by
titration with ISE detection and individual pCu measurements made at
t = 0, 24, and 48 h.

The study confirms the usefulness of the ISE measurements as detectors of changes in speciation
and as indicators of biological impact. The finding also attests to the primary role of CuCC in con-
trolling the health of the system. Therefore, the complexation capacity might be defined as the
amount of copper that can be added to the water without causing a harmful effect, and provides an
insight into the processes that control water quality in San Diego Bay.

40

WETLAND PLANTS' ROLES IN UPTAKE AND TRANSPORT OF HEAVY METALS
(P. Weis and J. S. Weis)

Wetlands can be used to mitigate pollution runoff and to bioremediate contaminated soils. Divalent metals become bound to organic matrices and become highly insoluble sulfides in the subsurface anoxic zone. The solubility product of copper sulfide (CuS), e.g., is $\sim 10^{-46}$. However, wetland plants can subsequently act as sources, as well as sinks of contaminants in an ecosystem. In order to thrive in anoxic, waterlogged soils, plants pump oxygen into their root zones. This action changes the redox status, and, in association with chelating organic root exudates, facilitates solubilization of metals.

The dominant vascular plants in northwest Atlantic estuaries are the saltmarsh cordgrass, *Spartina alterniflora* and the common reed, *Phragmites australis*. We have been studying contaminant flux and uptake/trophic transfer of metals, both in situ and in plants and detritus brought into the laboratory from natural and restored *Spartina* marshes and from *Phragmites* marshes. Our hypothesis that *Spartina* would export more metals into the water column via excretion through salt glands was proven: *Spartina* was found to excrete significantly more of all metals through leaf tissue than *Phragmites* under both field and laboratory conditions. *Spartina* was also found to accumulate significantly more chromium (Cr) and lead (Pb) in leaves than *Phragmites*. Therefore, *Spartina* both removes more of certain metals from sediments and excretes more of all analyzed metals into the water column. For Cu in particular, *Spartina* can excrete 125 g ha^{-1} d^{-1} (4.58 kg ha^{-1} yr^{-1}).

When leaves are shed in the fall, they fall to the marsh surface where microbial processes turn them into detritus, the base of the estuarine food web. Experiments were designed to determine which plant species breaks down sooner as detritus and releases its metal burden into the environment. Nylon mesh bags containing leaves from *Phragmites* and *Spartina* (the latter from both natural and restored marshes) were placed in two areas of the Hackensack Meadowlands, a major estuary in the most urbanized part of New Jersey, 4 to 10 km west of New York City. As seen in Figure 28, detritus continues to accumulate metal from contaminated sediment, exceeding the high sediment levels at one site but not the other. The site with higher uptake of Cu in detritus had a lower sediment pH than the other site. At both sites, the continuing uptake of Cu greatly exceeded the starting level in the leaves and stems, making it more biologically available. Detrital uptake of nonessential metals (Cr, mercury [Hg], Pb) were also high but did not exceed the sediment levels in the low pH site.

In summary, sediments are good at sequestering divalent cations such as Cu, but biological processes, including vascular plant growth and detritus formation, can return the sequestered Cu to a biologically available status.
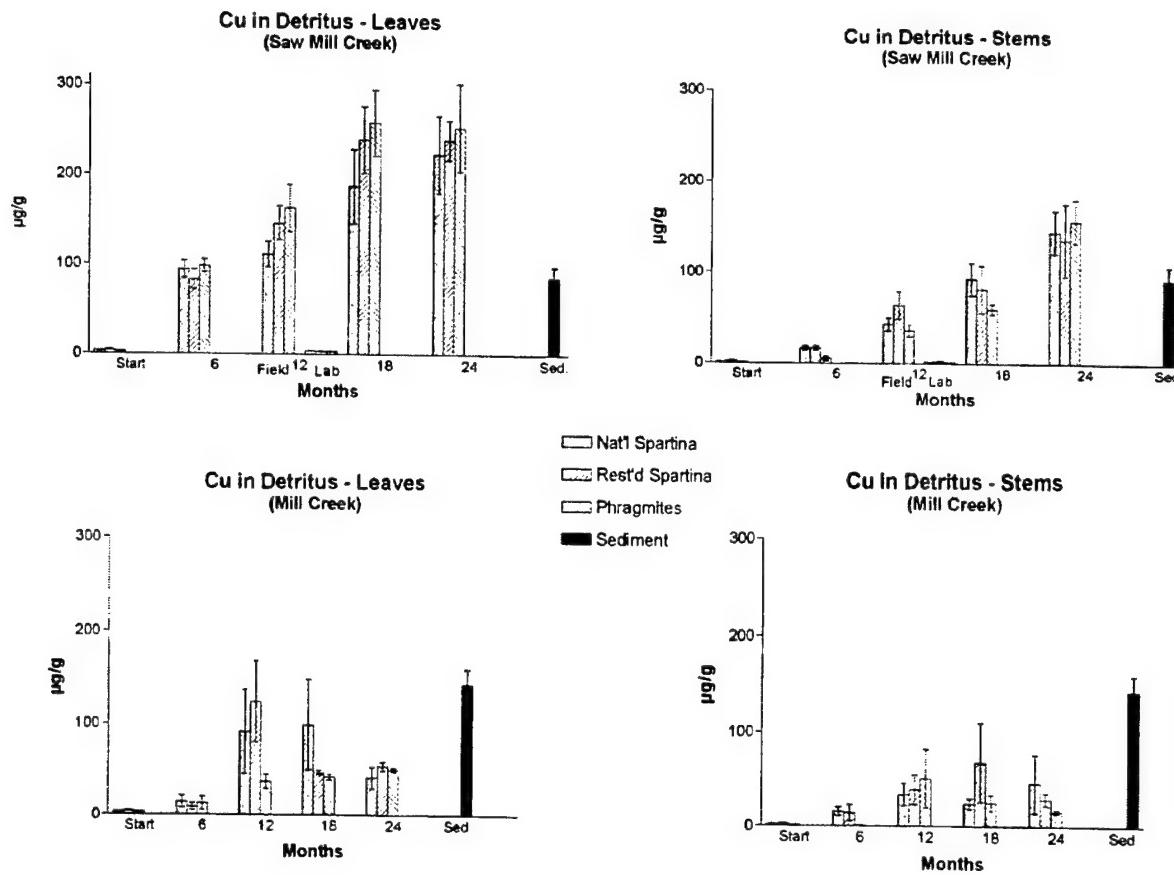
41

Figure 28. Copper levels in detritus forming in mesh bags from leaves and stems of *Phragmites* and *Spartina* (the latter from both natural and restored marshes) at two sites in the Hackensack Meadowlands, northern New Jersey.

DEFENSES

INTERACTIONS OF BIOPOLYMERS WITH CU AND OTHER HEAVY METALS IN GALVESTON BAY, TEXAS (P. H. Santschi, D. Tang, C.-C. Hung, K. Warnken, and M. Quigley)

Introduction

Colloids (particles so small [1 nm to 1 μm] that they never settle out of solution) play an important role in the regulation of trace-metal activity in the marine environment. Most B-type and many A-type and transition metals in estuarine waters are found to be complexed to natural organic matter (NOM) to a significant degree and more than 50% of NOM is composed of colloidal macromolecular organic matter (COM). Major biopolymers in COM with strong functional groups include extra-cellular acid polysaccharides containing carboxylic functional groups, and extracellular proteins containing thiolic and amino functional groups. These biomolecules can be produced by micro-organisms in response to nutrient and trace-metal stress. Thus, thiols and acid polysaccharides have a special role as metal sequestering and detoxification agents regulating metal concentrations, as well as bioavailability and toxicity. Colloidal particles obtained by ultrafiltration and detected with a transmission electron microscope (TEM) and by atomic force microscopy (AFM) are shown in Figure 29 (Santschi et al., 1998).

SCALE BAR = 500 nm   SCAN SIZE = 5 μm x 5 μm
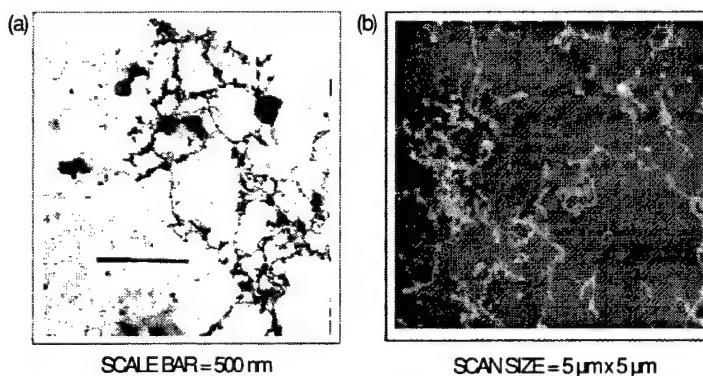
Figure 29. TEM (a) and AFM (b) images of surface water sample from Gulf of Mexico. The techniques give complementary but not identical information about colloidal size and shape distribution.

This work presents recent results from an investigation on the distribution of Cu and some other heavy metals, thiols, and acid polysaccharides in Galveston Bay, Texas. The long-term goals of this study are to accurately describe how and how rapidly trace elements in the marine environment become partitioned among physically different species. This goal is illustrated in Figure 30.
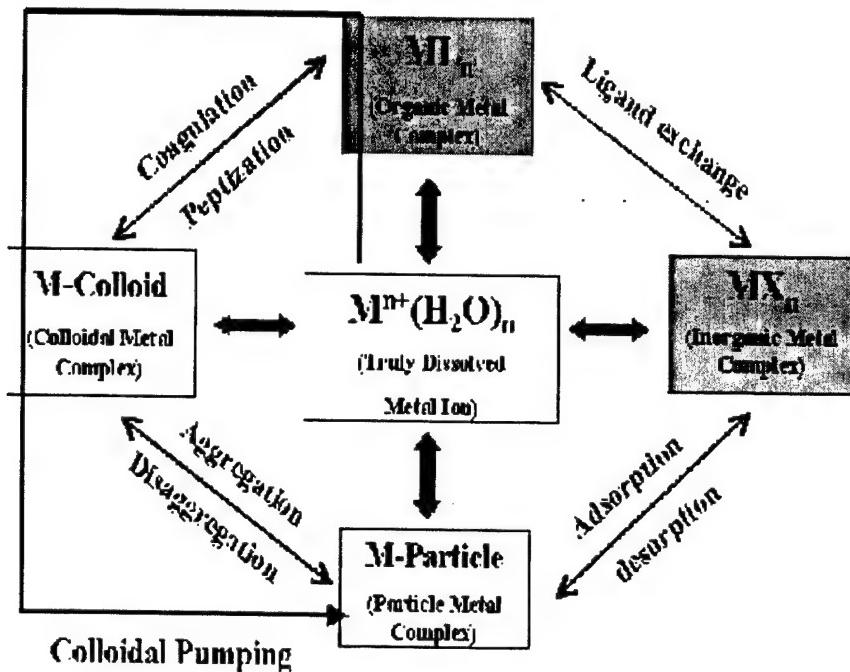
Figure 30. Interactions among metal species.

Major questions that need to be answered by research include the following: (1) Are the ligand groups of low or high molecular weight (HMW), namely, are they associated with a macromolecular backbone? (2) Are ligand groups "sheltering" complexed metals? (3) What are the HMW surface-active ligands? (4) Is the "ligand soup" part of a self-regulating (autoporetic) system?

In a purely oceanic environment, trace-metal binding ligands are produced by the phytoplankton externally through the production of polysaccharide-rich fibrils that bind group A and transition metals, and internally, as phytochelatins (large proteins containing sulfhydril [thiol] groups). The latter bind group B and transition metals. Both of these metal-binding ligands are released into the water column during the phytoplankton life-cycle. Thereafter, bacteria become instrumental in modifying this material.

Experimental

For this study, we collected samples throughout the estuarine regions of Galveston Bay by using trace-metal-clean techniques. The natural organic matter was separated into dissolved, colloidal, and particulate fractions by cross-flow ultrafiltration (CF-UF) followed by dialysis (Guo et al., 2000a,b; 2001). The Cu-ligand affinity was then characterized in each fraction according to the scheme shown in Figure 31.
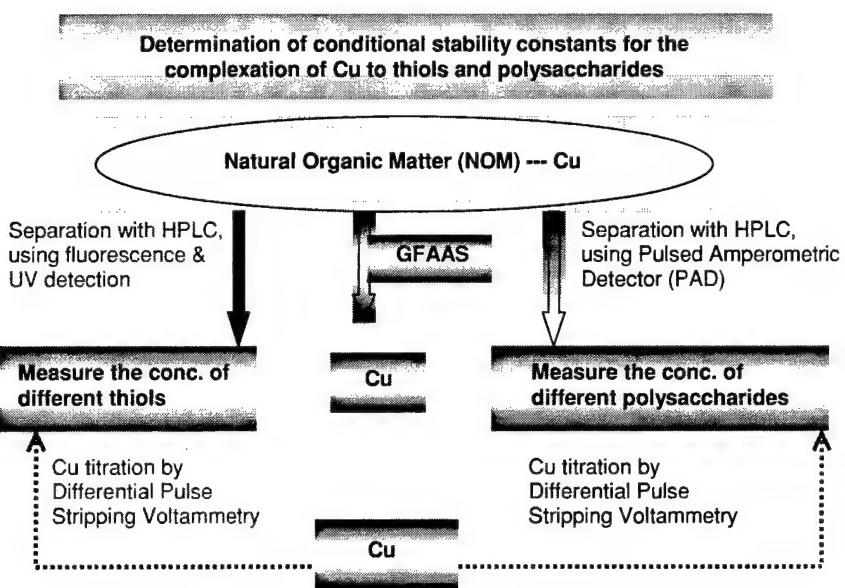
Figure 31. Analytical scheme for the characterization of NOM.

The experimental methodology is outlined in Table 4 below.

Table 4. Analytical methods.

Analyte	Method	Reference(s)
Copper	ICP-MS, DPCSV in the presence of catechol by competitive ligand equilibration / adsorptive cathodic stripping voltammetry	van den Berg, 1984; Tang et al., 2001)
Total Reduced Sulfur	Voltammetry	Tang et al., 2001
Thiols	HPLC	Tang et al., 2000a,b
Total Dissolved Sulfides	Spectrophotometry and HPLC	Tang and Santschi, 2000
Polysaccharides, Uronic Acids	Spectrophotometry	Hung and Santschi, 2001; Hung et al., 2001
Thorium	LSC, 2D-PAGE, UF	Quigley et al., 2001, 2002

Results

Major results of this study were that strong relationships exist among the concentrations of dissolved Cu (and Ni), chlorophyll-a (chl.-a), selected thiols, uronic acids, and total reduced sulfur —a strong indication that the metal-binding ligands originate from the plankton (Figure 32).

45
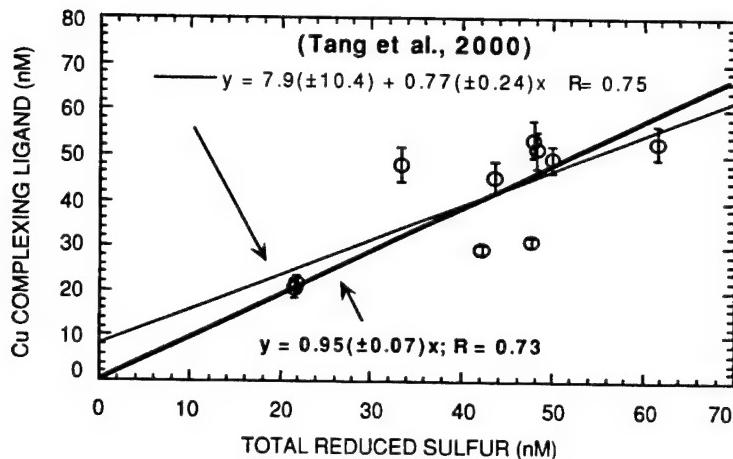
Figure 32. Relationship between total reduced sulfur and Cu-complexing ligand.

Additionally, polysaccharide NOM showed a higher metal binding affinity than bulk colloidal organic matter, indicating that polysaccharides could also be the source of metal-binding ligands.

Conclusions

Both acid polysaccharides and thiols are good candidates for the colloidal HMW surface-active metal-binding ligands. As deduced from experiments with copper and thorium, important ligand groups are of HMW and likely associated with a macromolecular backbone. It is suggested that the ligand groups must be "sheltering" at least some complexed metals, viz., rendering them unavailable for simple exchange reactions. In this sense, an egg-crate model is suggested for the structure of the metal-HMW-binding ligands. Finally, the "ligand soup" is regulated, in part, by the microbial community in the water, and under the right conditions, is likely to be a self-regulating (autoporetic) system.

RECENT ADVANCES THAT POSE NEW PROBLEMS FOR REGULATORS (J. Moffett)

In recent years, the U.S. EPA and other regulatory agencies have adopted a progressive approach to the regulation of Cu; the adoption of site-specific criteria and WERs are good examples of this approach. However, this work is an ongoing process, and new developments suggest that some paradigms about Cu chemistry and toxicity may require modification. Some of these developments are driven by new science. For instance, there is accumulating evidence that reduced sulfur ligands may be important Cu chelators. Since reduced sulfur ligands are prone to oxidation, but Cu(I) complexes are very strong, the finding suggests that kinetic rather than thermodynamic controls may dominate Cu speciation. Variability in the sources and sinks of these species may lead to greater temporal and spatial variability in Cu bioavailability than we have currently considered. Also, measurements of free cupric-ion concentrations, determined from titration data assuming thermodynamic equilibrium and Cu(II) dominated chemistry may need to be reassessed. Other developments are driven by the regulatory focus on episodic events, particularly episodic discharges from point and nonpoint sources associated with runoff events. These events are a major issue at Navy bases, as discussed in the previous workshop.

PRODUCTION OF COPPER-COMPLEXING LIGANDS BY BACTERIA IN CULTURE AND BY INTACT MICROBIAL COMMUNITIES IN SITU (A. Gordon)

High-affinity, copper-complexing ligands control the speciation and, consequently, the bioavailability and toxicity of copper in marine and estuarine waters. There are a number of ways that microorganisms protect themselves against metal toxicity, including (1) intracellular sequestration, (2) the direct release of the metal in the medium, and (3) by ligand secretion. Intracellular sequestration occurs in a number of ways. A principal avenue is via the production of metallothioneins, viz. metal-binding proteins with thiol (S-H) functional groups in cyanobacteria (*Synechococcus*) and many higher organisms, and by production of phytochelatins (a class of metallothioneins) in microalgae, yeast, fungi, and higher plants.

A second path, possibly of lesser importance, is via the formation of inorganic complexes (e.g., phosphates) by bacteria (*Citrobacter*) and periplasmic and membrane proteins by bacteria (*Pseudomonas syringae*). Efflux of metals by bacteria is accomplished through energy-dependent ion pumps (*Enterococcus, Vibrio*) and periplasmic and membrane proteins (*E. coli*). Finally, copper-induced, ligand excretion yields yet uncharacterized metal-binding compounds (*Synechococcus, Vibrio*), Cu-complexing proteins (*Vibrio*), siderophores (*Cyanobacteria*), and nonspecific exopolysaccharides and proteins.

It is believed that the production of uncharacterized L_1 class ligands is a Cu detoxification mechanism with major ecological implications because (1) they generally occur in the water column at concentrations similar to dissolved copper (10^{-8}M to 10^{-9}M), (2) have extremely high affinity for copper (stability constant; log K' $_{Cu}$ 2+ = 12-14), and (3) have been detected and measured in all types of marine waters. Indeed, dissolved copper in most marine and estuarine waters exists as the L_1 complex.

What we don't know about L_1 class ligands includes in-situ sources and sinks, the molecular structures, the mechanisms of actual complexation, and the actual physiological mechanisms of production. It is now established that various types of bacteria in culture excrete ligands with copper-binding characteristics similar to the L_1 ligands present in seawater (Figure 33).
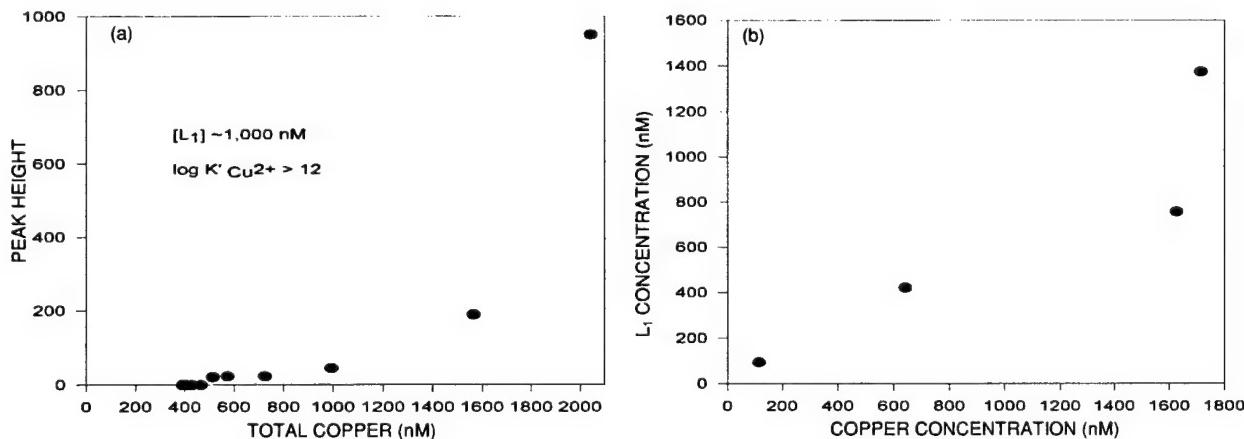
Figure 33. Production of L_1-class ligands in (a) a copper-stressed (390 nM) *Synechococcus* (PCC 73109) chemostat and (b) in a culture of *Vibrio alginolyticus*.

Ligand concentration in bacterial cultures has been demonstrated to increase with increasing copper concentration, strongly supporting the suggestion that ligand production serves as a copper detoxification mechanism for the bacteria.

Recent experiments using intact microbial communities exposed to elevated copper levels in situ indicate that ligand excretion in response to copper stress also occurs in the estuarine environment at realistic copper concentrations (Figure 34). Thus, ligand excretion by bacteria is likely to responsively alter copper speciation and bioavailability on relatively short time scales.
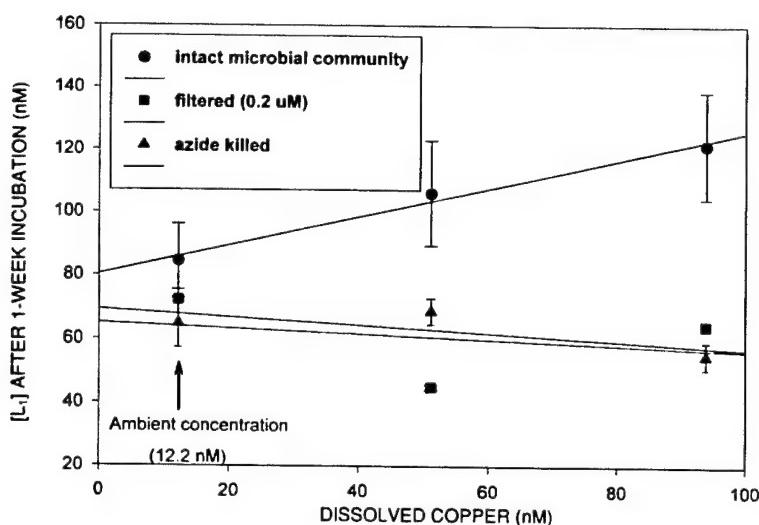
Figure 34. In-situ incubation at the Norfolk Naval Station: Ligand concentration in treated and untreated water samples at varied copper concentrations.

In summary, it is clear that diverse copper detoxification mechanisms exist and that L1-class ligands of unknown character dominate speciation of dissolved copper. Microorganisms appear to be a source of these ligands. In an in-situ experiment, ligand production in response to copper addition was observed within 1 week. Ligand production was not observed when the microbial community was removed or killed. The process of ligand export is ecologically important because it may affect the entire community.

COPPER COMPLEXATION, SPECIATION, AND SEDIMENT-WATER CYCLING IN A MAJOR U.S. HARBOR (ELIZABETH RIVER, VA) (J. R. Donat and D. J. Burdige)

The Elizabeth River (ER)/Hampton Roads Harbor (HRH) is a major deep-water port and is the site of Norfolk Naval Base (NNB), the world's largest naval base and home of the U.S. Atlantic Fleet. ER/HRH is directly adjacent to Chesapeake Bay, the largest and historically most productive U.S. estuary. The ER watershed is among the most heavily urbanized and industrialized areas in the eastern U.S. The ER is also the location of U.S. Navy and private shipyards, as well as fuel, oil storage, and creosoting facilities, and chemical and fertilizer companies. Sediment concentrations of Cu and other metals in the industrialized portions of the ER are enriched relative to crustal abundances. In 1983, the U.S. EPA's Chesapeake Bay Program identified the ER as one of the most heavily polluted bodies of water in the Chesapeake Bay watershed; consequently, the ER was designated a "Region of Concern," a delineated area found to be, or with the potential to be, adversely impacted by chemicals.

In conjunction with other ONR Harbor Processes investigators, we are performing an integrated study of the biogeochemical cycling of Cu (and other metals) in the water column and sediment porewaters of the ER (Figure 35). We are studying the interrelationships among (1) trace-metal concentrations, complexation, and speciation (Donat); (2) in-situ production of Cu chelators by natural microbial populations (in collaboration with Gordon/Donat [Old Dominion University]); (3) phytoplankton metal uptake (in collaboration with Sunda/Huntsman [National Oceanic and Atmospheric Administration (NOAA)/National Marine Fisheries Service (NMFS)]); and (4) fluxes of metals and chelators from sediments (Donat and Burdige).
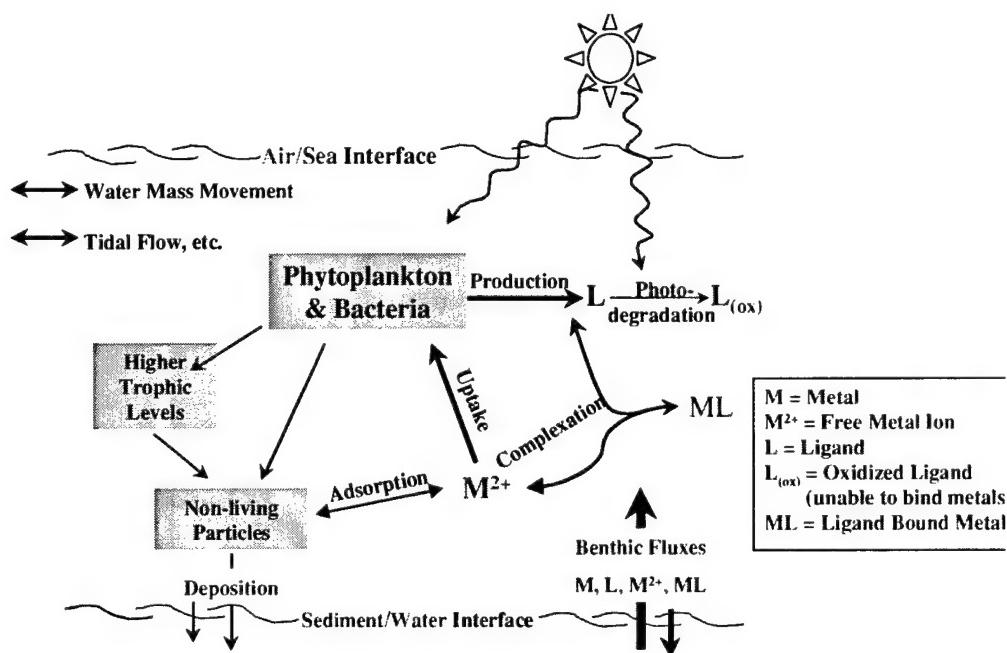
Figure 35. Metal-environment inter-relationships being studied in the Elizabeth River.

In July 1999 and May 2000, water samples were collected at several stations starting from Chesapeake Bay and going upstream into the Elizabeth River (Figure 36). Total metal was measured with APDC/DDDC chelation-extraction followed by detection by GFAAS. Measurements of metal complexation were made using voltammetric techniques (ligand exchange followed by cathodic stripping voltammetry). Water-column results from our two major ER field studies indicate that concentrations of total Cu (unfiltered: Cu_T) increased upriver from 7 to 9 nM in HRH to 60 to 80 nM (near Norfolk Naval Shipyard, [NNSY]), then decreased further upriver to 60 nM. Total dissolved Cu (0.22 µm-filtered: Cu_{TD}) ranged from 6 to 7 nM in HRH to 50 nM near NNSY.
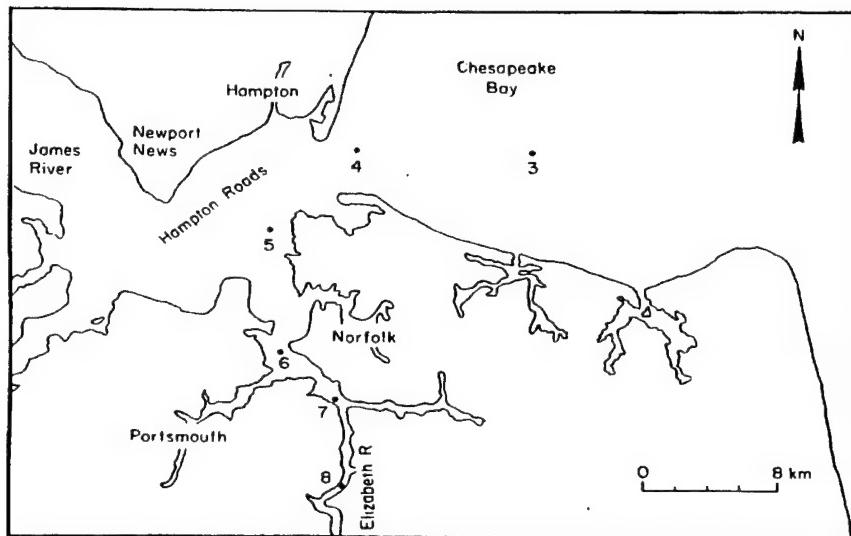
Figure 36. Locations of stations for Elizabeth River contamination study.

At most stations, there were no significant differences in Cu_T or Cu_{TD} between July 1999 and May 2000. In all surface-water samples, Cu_{TD} was >99.9% complexed by one strong organic ligand class, L (average log K_{Cu2+}'= 12.2), whose concentration ([L]) ranged from 35 to 54 nM in HRH, increasing upriver to 100 to 200 nM (Figure 37).
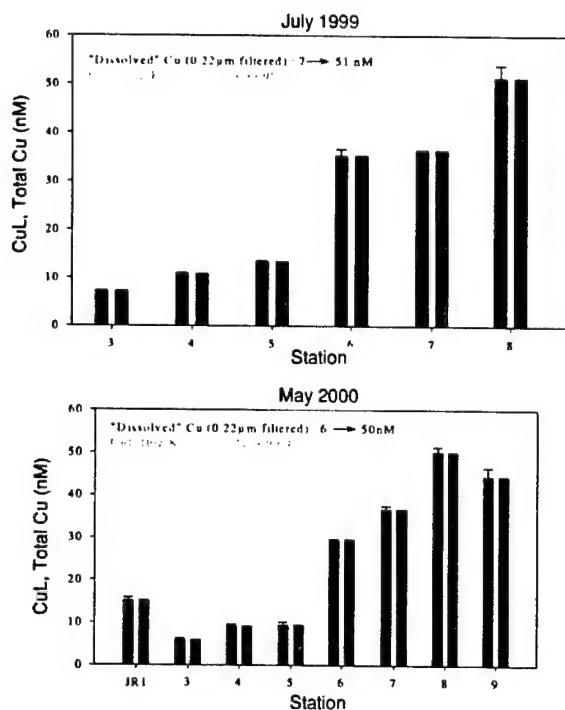
Figure 37. Copper complexation at measured stations, Chesapeake Bay–Elizabeth River. (Dark bars: dissolved Cu_{TD}; light bars: CuL)

50

[L] exceeded Cu_{TD} by 2 to 10x at all stations. In May 2000, the resulting free Cu^{2+} ion concentrations ([Cu^{2+}], the potentially toxic form, ranged from 0.042 to 1.9 pM (pCu range: 13.4 to 11.7); however, they were less variable in Figure 37. Copper complexation at measured stations in July 1999 were ([Cu^{2+}] range: 0.1-0.6 pM; pCu range: 12.2-12.9). All [Cu^{2+}] were below the concentrations reported to reduce the growth rate of Cu-sensitive ciliates and the survival rate of the naupliar stage of a common estuarine copepod. Thus, while Cu_{TD} increased upriver by 10x, the potentially toxic [Cu^{2+}] increased upriver by as much as 40x. However, even the highest [Cu^{2+}] concentrations we measured were below reported toxic levels.

We have performed benthic-flux studies at two sites in the ER: Stn 5 (near NNB) and Stn 8 (near NNSY), and at two sites in the mainstem of Chesapeake Bay (CB). Our data indicate that CB sediment porewaters contain concentrations of copper-complexing ligands that exceed those in the water column by 15x – >100x. The flux of these copper ligands out of the porewaters exceeds the flux of Cu_{TD} by 3 to 40x. This ligand flux may supply 10 to 50% of the water column standing stock of copper ligands in CB. In addition, the results of laboratory photochemical degradation experiments suggest that porewater-derived copper ligands can persist in the CB water column with residence times of ~140 days, which is significant compared to the residence time of water in CB of 180 to 360 days.

In ER, measurable fluxes were only observed for the stronger (L_1; log K' > 10) of two Cu-complexing ligand classes. In general, both Cu_{TD} and L_1 fluxes were higher near NNSY than near NNB. This is likely a result of both higher sediment metal concentrations near NNSY as well as overall higher rates of sediment organic matter remineralization near NNS. Cu_{TD} fluxes near NNSY were >100x greater than those observed at our CB sites, and the L_1 fluxes sometimes appeared to be uncoupled from total metal fluxes. For example, near NNB, we saw essentially no Cu_{TD} flux but rather a large L_1 flux, comparable to that which we observed near NNSY. We observed a similar uncoupling between Cu_{TD} and L_1 fluxes at our CB sites.

THE ION BALANCE MODEL (IBM): A PHYSIOLOGICALLY BASED EXTENSION OF THE BIOTIC LIGAND MODEL (BLM) (P. Paquin)

The biotic ligand model (BLM) of the acute toxicity of metals has been developed to predict the toxicity of waterborne metals to aquatic life (USEPA, 1999; Di Toro et al., 2001). Within the context of this mechanistically based framework, the "biotic ligand" corresponds to binding sites at the site of action of toxicity, the tissue (i.e., the gill in fish), or a subset of physiologically active sites at that tissue where metal accumulation results in the manifestation of a toxic effect. The biotic ligand is represented in the same way as any other ligand in solution, having a characteristic binding site density and a conditional stability constant for each of the dissolved chemical species with which it reacts. The BLM, based on a chemical equilibrium computational approach, simultaneously accounts for the speciation and complexation of dissolved metal in solution and competition among the available metal forms, typically the free metal ion and other cations, for binding sites associated with biotic ligand. Previously, the 96-hour LC50 has been associated with a critical level of metal accumulation at the biotic ligand (the lethal accumulation at 50% mortality, or LA50) and the BLM predicts the LC50 in different waters by predicting the concentration of dissolved metal that must be present in association with this LA50. The BLM is currently under consideration by the U.S. EPA for use in the development of updated WQC for metals (USEPA, 1999). The original implementations of the BLM were developed for use in the evaluation of copper and silver toxicity to freshwater organisms (Santore et al., 2001; Paquin et al., 1999). The copper BLM has subsequently been applied to the sensitive saltwater mussel, *Mytilus edulis* (Di Toro et al., 2000). The mechanistic underpinnings of the BLM for silver have been described (McGeer et al., 2000), and several other applications of

the BLM to other metals have been reported (e.g., De Schamphelaere and Janssen, 2002; De Schamphelaere et al., 2002; Heijerick et al., 2002; Santore et al., 2002).

The BLM applications described above did not explicitly consider the physiological effects of the metal on the organisms, nor did they consider the direct effect of water-chemistry parameters such as Ca^{2+}, pH, and Na^+ on the physiological state of the organism. Recently, a physiologically based ion balance model (IBM) of survival time has been developed that makes use of the BLM to evaluate the degree of accumulation of the metal at the site of action of toxicity (Paquin et al., 2002). The model includes an evaluation of the physiological response of the organism to exposure to the metal and to the water-chemistry characteristics as well. The initial implementation of this model for rainbow trout, exposed to silver in freshwater, and the potential for application of this same model framework to copper, including copper exposures in salt water, are discussed below.

The initial IBM implementation described here is based on a mass balance of sodium ions, Na^+, about the organism, in this case, a fish (Figure 38).

GILL/BIOTIC LIGAND
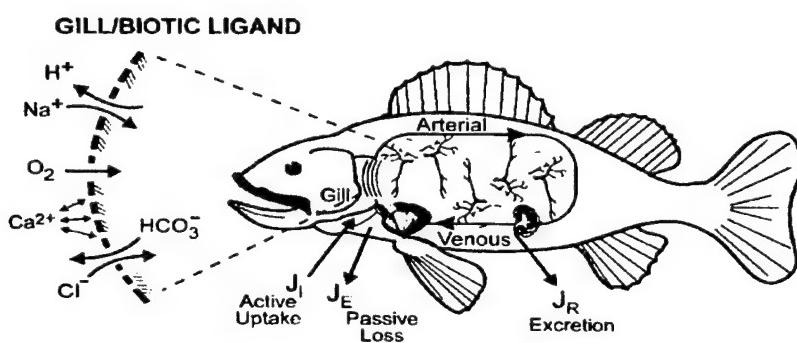
Figure 38. Illustration of the ion balance model applied to sodium regulation by a freshwater fish.

The basic idea is that, under normal conditions, the exchangeable sodium pool (contained within the internal fluid compartments of the fish) is in equilibrium with the external water such that the plasma sodium level is constant over time. However, exposure to a metal such as silver or copper will inhibit the active (i.e., energy-requiring) uptake of sodium from freshwater (or the active efflux in seawater). This exposure results in a net imbalance in ion fluxes, where the sum of renal losses plus passive diffusion losses at the gill (the net passive diffusion flux is outward in freshwater and inward in seawater) exceeds active uptake at the gill, and the fish begins to lose sodium. The rate of loss depends on the degree of inhibition of Na^+ uptake, which the IBM relates directly to the level of metal accumulation, as calculated by the BLM. Regardless of either the specific stressor that is causing the ionoregulatory disturbances or the duration of exposure, a biomarker that is often associated with lethality in freshwater fish is an approximate 30% depletion of plasma sodium levels from the norm (McDonald et al., 1980; Wood, 1989; Wood et al., 1996; Webb & Wood, 1998; Hogstrand & Wood, 1998; Grosell et al., 2000). Although lethality does not result from loss of sodium alone, and the use of a 30% loss is at best approximate, association of a set plasma sodium level with lethality serves as a convenient biomarker for modeling purposes. The physiological effect of Na^+ is represented via a Michaelis expression for sodium uptake, which is a function of the maximum sodium uptake rate and the sodium concentration of the external water. The physiological effect

of Ca^{2+} is represented by a relationship with gill permeability, where the increase in permeability that results from physical damage to the gill epithelium being mitigated (i.e., permeability decreases) as calcium increases. At the same time, the degree of the effect of exposure to the waterborne metal on sodium uptake rate is quantified via a dose–response relationship between inhibition of the maximum sodium uptake rate and the BLM-predicted biotic ligand silver concentration. The physiological effect of pH has been neglected since it was not varied for the circum-neutral pH test conditions considered to date. The mass-balance equations for each of the internal-fluid compartments that are represented in the model and a more detailed description of how the model was applied are presented elsewhere (Paquin et al., 2002).

The general approach to applying the IBM is as follows. The model is initially calibrated to a data-set where rainbow trout were exposed to 3.2 µg/L of silver, while chloride was varied, and plasma sodium levels of the fish were monitored over the ensuing 48 hours (McGeer & Wood, 1998). The variation of chloride levels is important, as it forms silver chloro-complexes, primarily AgCl, and this form of silver has been shown to markedly reduce silver bioavailability to rainbow trout. In the context of the silver BLM, at a fixed dissolved silver concentration, when the chloride concentration is low, silver availability is high, and the predicted BL:Ag will be high. Then, as the chloride level increases, silver availability decreases, resulting in a decrease in BL:Ag. The BL:Ag is evaluated with the previously developed silver BLM (Paquin et al., 1999) with the toxicity test water chemistry specified as inputs for each of the treatments. The parameters of the dose– response curve, the EC50 for uptake inhibition, and ∃, which characterizes the slope of the response, are adjusted by calibration to the observed response in plasma sodium data. The results of the IBM simulation analysis are compared to the rainbow-trout plasma-sodium data on Figure 39. The plasma-sodium time-series data (+/- standard error) are shown for the controls (Ag = 0) on Figure 39a. Some unexplained variability is evident, as control-fish plasma-sodium levels would be expected to remain approx-mately constant over the 48-hour test duration. These changes are likely to be within the range of normal physiological variation and may also reflect sampling and analytical variability. With regard to the model results (the solid line), the initial condition was set equal to the average concentration over the test duration, and it remains constant in time. The gill permeability was evaluated such that the uptake and loss terms were in equilibrium, and since the BL:Ag = 0, there is no inhibition of sodium uptake for the control fish.

Figures 39b through 39d present comparisons of representative model results to the plasma-sodium data for three of the remaining five 3.2 :g/L dissolved Ag treatments. Results are presented in order of decreasing chloride (1440 :M to 14 :M) and increasing predicted BL:Ag (3.4 to 12 nmol/g_w). At the highest chloride level (1440 :M; Figure 39b), the predicted BL:Ag = 3.4 nmol/g_w, resulting in less than 1% uptake inhibition. Thus, the decrease in plasma-sodium concentration relative to the initial condition (set to the average of the initial and 1-hour measurements) is negligible over the 48-hour test duration, well within the limits of the measured plasma-sodium levels. As chloride levels are progressively decreased in the remaining treatments (Figures 39c & 39d), a clear pattern of decreas-ing plasma-sodium levels (increasing losses of sodium) is evident in the data. Since the predicted BL:Ag also increases with decreasing chloride levels, resulting in an increasing degree of inhibition of sodium uptake, the model results follow the same trend as the data, with progressively higher losses of sodium over time as chloride levels decrease. The fit of the plasma-sodium data of Figure 39 was achieved by assigning an EC50 for inhibition of J_M = 15.8 nmol/g_w in association with a slope for the dose– response curve of ∃ = 0.278, as well as intercompartmental permeability coefficients of 0.1 L/kg_w/d. (Although the IBM computes sodium levels in each of four internal-fluid compartments, only plasma-sodium levels are shown here.)
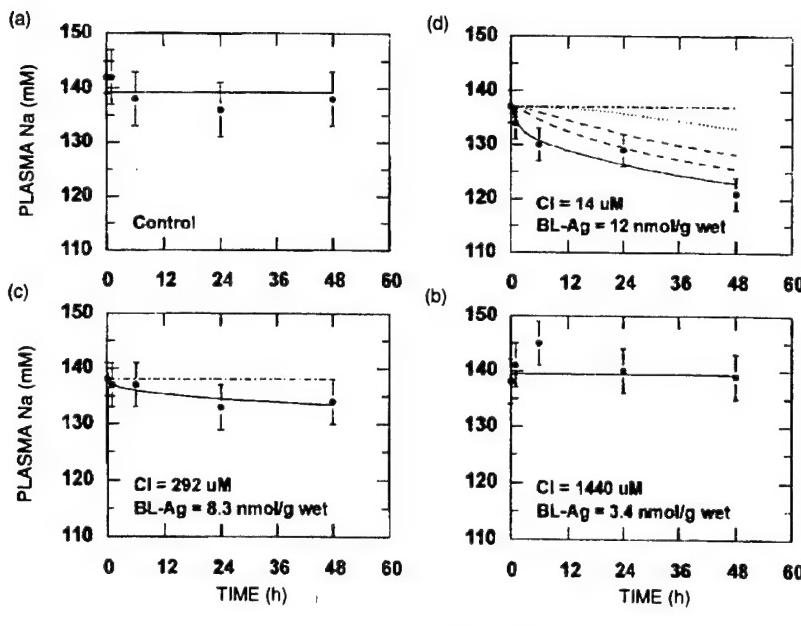
Note: Ag_T = 3.2 ug/L except Control
Data: McGeer and Wood, 1998

Figure 39. Comparison of rainbow-trout plasma-sodium time-series data (+/- standard error) to model results for control fish (Ag = 0) and fish exposed to 3.2 :g/L of dissolved Ag, as Cl⁻ varies (McGeer & Wood, 1998).

The results of Figure 40 demonstrate the ability of the sodium IBM to predict plasma-sodium levels over time. This capability is important since it is a simple matter for the computations to be continued until the critical plasma-sodium concentration associated with lethality is reached (the time when that occurs being the predicted survival time). This was done for a second set of experimental results where rainbow trout were exposed to a very high concentration of 100 µg/L silver, and calcium was varied from 0.05 to 5 mM/L by adding either $Ca(NO_3)_2$ or $CaSO_4$, or chloride was varied by adding NaCl or KCl (Galvez & Wood, 1997). As shown in Figure 40, these treatment conditions resulted in survival times that varied from less than 1 hour to more then 7 days, and the model was able to predict this range of results quite well. Consistent with the observations, the predicted survival times increased with increasing calcium from less than 1 hour to almost 12 hours, and the survival times were not sensitive to the anion, NO_3^- or SO_4^{2-}, that was added in association with the Ca^{2+} additions.

The IBM also responded in accordance with the results for the chloride treatments, with survival time increasing with increasing chloride from less than 1 hour to more than 7 days, and with somewhat longer survival times associated with the addition of sodium in comparison to potassium. This finding was, in part, due to the competitive effect of sodium at the biotic ligand, but, even more so, it was due to the fact that the added sodium facilitated the uptake of sodium via the Michaelis term that was used to represent the kinetics of sodium uptake.

While the preceding application of the IBM was for exposure of rainbow trout to silver in freshwater, it is expected that the same approach is applicable to other forms of aquatic life, including

54

sensitive invertebrates, in both freshwater and saltwater, and to other metals as well, including copper. With regard to copper, the mechanisms of copper toxicity to aquatic life are, in many ways, similar to the mechanisms of silver toxicity (Wilson & Taylor, 1993a; Morgan et al., 1997; Wood, 2001). Of particular importance is the observation that both inhibit the activity of sodium-potassium ATPase (NKA), an enzyme that is directly related to the active regulation of sodium levels in fish and other forms of aquatic life, and, at sufficiently high levels, they may also affect gill permeability. Stagg and Shuttleworth (1982) demonstrated that the NKA activity of gill homogenates of saltwater-adapted *Platichthys* is suppressed by exposure to Cu in saltwater and that the response occurs in a dose-dependent manner. The response was somewhat less severe in Cu-acclimated fish (200 µg/L Cu for 42 days).
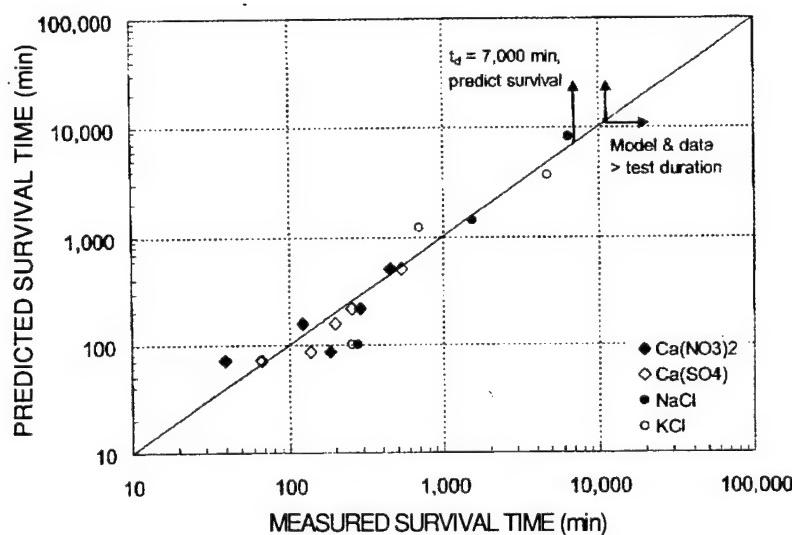
Figure 40. Comparison of measured versus predicted survival times for rainbow trout exposed to approximately 100 µg/L Ag and varying levels of calcium and chloride (Galvez & Wood, 1997).

An important difference between the response to metals in freshwater and saltwater settings is that the external sodium levels are higher in saltwater, so the direction of the passive diffusion flux is reversed, and sodium and some other ions must be actively excreted rather than taken up. The other potential effect of Cu is on gill permeability. As an example, consider the results of Wilson and Taylor (1993b) who exposed rainbow trout to 60 uM Cu (~360 ug/L) in about 1/3 seawater and in full seawater. At 1/3 seawater, plasma sodium increased, while at full seawater, the response was reduced. Wilson and Taylor concluded that it wasn't so much an effect of NKA inhibition, but rather, at 1/3 seawater, Cu affected permeability of the gill and plasma sodium increased until it equaled the concentration in the external water. While at full seawater, the ambient conditions provided a protective effect on the gill, and hence there were no effects. Another important difference between freshwater and saltwater settings is that uptake of sodium (and chloride) from drinking water is also important in saltwater, resulting in a further need to actively excrete sodium at the gill. Hence, uptake from drinking water will probably need to be considered if the IBM approach is to be extended to saltwater conditions.

With regard to other organisms, Mantel and Farmer (1983), in an extensive review of plasma osmolality data for invertebrates, have shown that essentially all aquatic organisms must osmoregulate, though to varying degrees. The results they summarize suggest a similar IBM approach could be

applied to other forms of aquatic life. Mantel and Farmer (op. cit.) also suggest that sensitivity to metals should be reduced for organisms that are osmoconformers (plasma osmolality approximately the same as the external water) or weak osmoregulators (plasma levels are only slightly different from the external water), since the water-plasma ion gradients are reduced, and the need to actively excrete ions is also likely to be lower. This expectation is consistent with the results for rainbow trout in saltwater that were described previously (Wilson & Taylor, 1993b). It is also consistent with the results of other studies with freshwater fish where it has similarly been shown that increasing the ionic composition of the external water (i.e., increasing the osmolality) from levels in freshwater to levels approaching the plasma had a protective effect against ion loss (Packer & Dunson, 1970; Lewis & Lewis, 1971).

The IBM approach may not be appropriate in all cases, such as for the sensitive larval stage of *Mytilus edulis* where the mode of toxicity is not known to be of an ionoregulatory nature. However, the IBM application to sodium that is described herein does add a new physiological dimension to the previously developed BLM, one that should extend the use of the BLM to other areas of toxicological and regulatory interest. In addition to the already demonstrated capability of the IBM to predict effects in relation to exposure duration, this physiologically based framework should also have use in the analysis of time-variable exposures, residual after-effects of exposure to metals, acclimation, chronic toxicity, and species and genus sensitivity (Paquin et al., 2002). Each of these is of potential use to longer term ongoing efforts to develop and refine WQC for metals.

MODELING CONSERVATIVE AND NONCONSERVATIVE BEHAVIOR OF COPPER IN SAN DIEGO BAY (B. Chadwick, A Zirino, I. Rivera–Duarte, and C. Katz)

The behavior of copper in Navy harbors is regulated by a number of physical, chemical, and biological processes that control the fluxes of copper in and out of the bay. A model that successfully predicts the concentration of copper in the bay necessarily relies on an accurate mathematical representation of these processes. In this study, we evaluate the relative importance of physical mixing and tidal exchange versus sedimentation for the mass balance of total copper in San Diego Bay by using a simple mathematical model. We then further refine the model to include copper species (the physico-chemical forms of copper that may exist in the water column and at the sea-sediment interface) and interspecies transformations (dissolved to particulate) to arrive at a distribution of species. This distribution includes the biologically available copper (the copper activity), which is measurable in the field, and which provides the best measure of biological and ecological impact. This work is part of a larger Strategic Environmental Research and Development Program (SERDP) project to evaluate the ecological impact of copper and zinc in Navy-occupied harbors. The SERDP project relies heavily on the field-survey developed by SSC San Diego and preceding laboratories over the last 30 years. Figure 41 provides a short description of this asset.

A one-dimensional, steady-state, nonconservative model of the bay is "tuned" by using salinity and then applied to the copper balance by using the latest available copper loading data. Figure 42 provides a diagram of the model. It is a box model in which conservative properties (flushing) are described horizontally between the boxes and along the axis of the bay, while nonconservative properties are stipulated within the boxes.

Field Sampling Program

Sampling Program - Capabilities

- Specialized sampling boat and equipment for trace metals
- Integrated, real-time measurement & mapping of total Cu/Zn, Cu activity, CTD, GPS
- Composite regional sampling for speciation, bacteria/phytoplankton populations
- Complete capabilities for intact sediment cores, and interface sampling
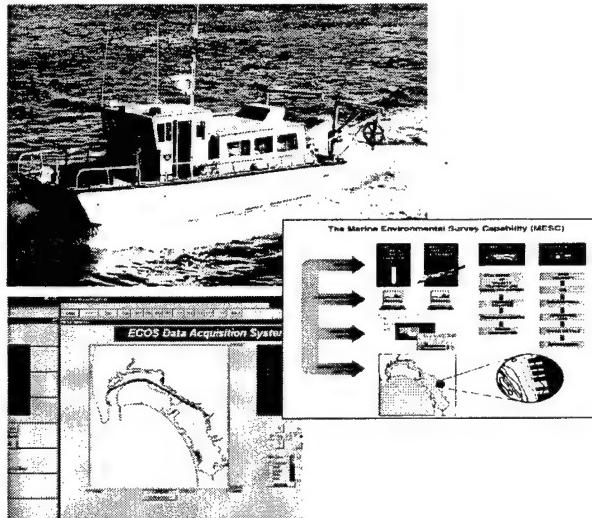
Figure 41. The SSC San Diego-developed Marine Environmental Survey Capability (MESC) system.

Model SD-1D – Steady State, Nonconservative

- Linked Box Model of San Diego Bay (Largier et al., 1997 and others)
- One or Two Dimensional (x or x-z)

- Tidal Exchange Calibrated to Conservative Tracer (Salt)
- Nonconservative Behavior Based on Partitioning
- Nonconservative processes occur within boxes - *partitioning, sources, sedimentation*
- Conservative processes occur between boxes - *tidal exchange, advection*
- Steady-state assumption should apply during predictable times of the year in San Diego Bay (Chadwick et al., 1995)
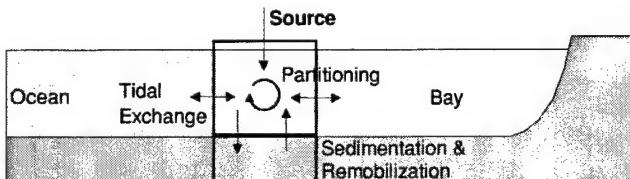
Figure 42. Schematic of the one-dimensional model in San Diego Bay.

Results from the model are evaluated in light of a recently collected model-matched field data set. The results indicate that conservative (flushing) and nonconservative (sedimentation) processes play roughly equal roles in the fate of total copper in the bay. Maximum total copper concentrations predicted with the conservative model are approximately four times higher than observed. The

nonconservative model estimates give good agreement with those measured with uniform, first-order loss term. The best fit to the data is with a loss (sedimentation) term of $R_{Cu}T=7.5\%$/day (Figure 43).
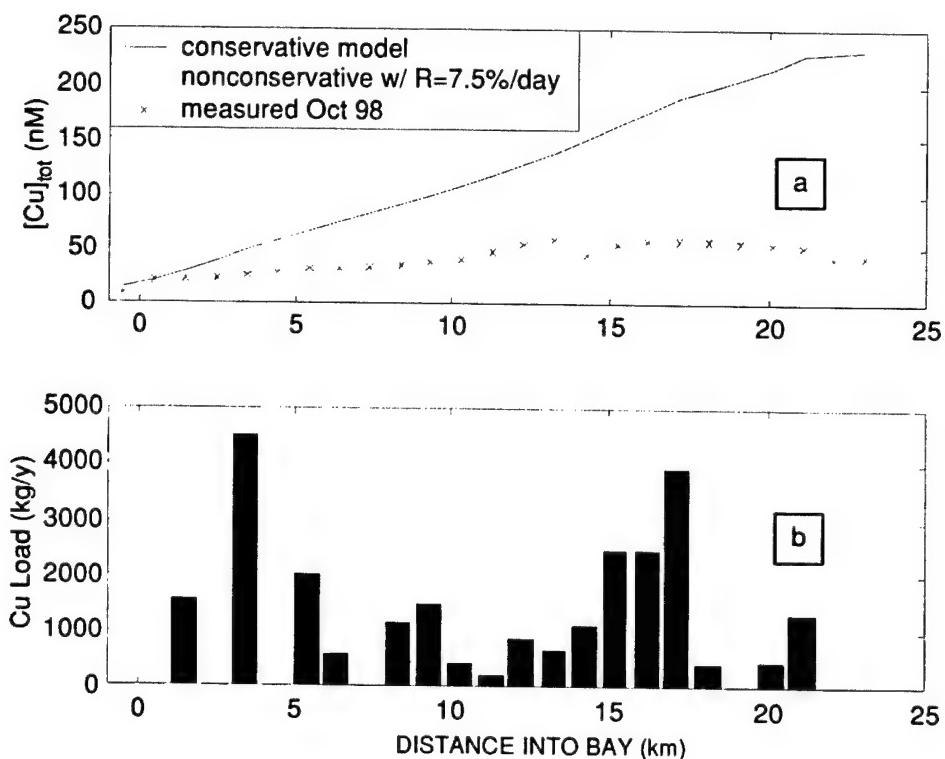
Figure 43. (a) The effect of nonconservative processes (sedimentation) on the copper distribution in San Diego Bay from mouth to head. Upper line is the conservative (no loss) modeling result, and lower line is the result using 7.5% loss per day compared to measured result. (b) Estimated total copper input along the same longitudinal axis.

Good agreement between nonconservative modeling results and field results is achieved by using a uniform, first-order loss coefficient. This factor allows comparison of copper carried to the sediment and copper flushed to the ocean. The model results are then further extended to evaluate the relationship between various copper species measured during the field study, including total copper, dissolved copper, and free copper ion. A simplified conceptual speciation model focuses on organic complexation (L), aggregation, and sedimentation. Complexation is presumed to be fast and approaches equilibrium (minutes to hours). Aggregation and sedimentation are presumed to be slower and to have the same time scale as flushing (hours to days). These transformations are shown below:

$$(Cu^{++})_{aq} + L \iff (CuL)_{diss.} \iff (CuL)_{coll.} \iff (CuL)_{part.}$$

$$(CuL)_{sediment}$$

58
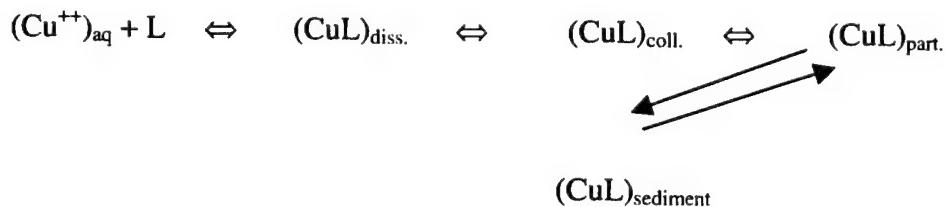
We used the measured values of [Cu]free and [L] (at a few points). Equilibrium between [Cu]free, [L], and [CuL]diss. is assumed. The species predictions are critically dependent on knowing the properties of L, which, at this moment, can only be measured.

Figure 44 depicts some of the results of the interspecies model.

[CuL]$_{diss}\rightarrow$[CuL]$_{coll}$

R = 19.8 %/day

T$_{1/2}$ = 3.5 days

[CuL]$_{diss}$+[CuL]$_{coll}\rightarrow$[CuL]$_{part}$

R = 10.8 %/day

T$_{1/2}$ = 6.4 days

[CuL]$_{diss}$+[CuL]$_{coll}$+[CuL]$_{part}\rightarrow$[CuL]$_{sed}$

R = 7.5 %/day

T$_{1/2}$ = 9.0 days
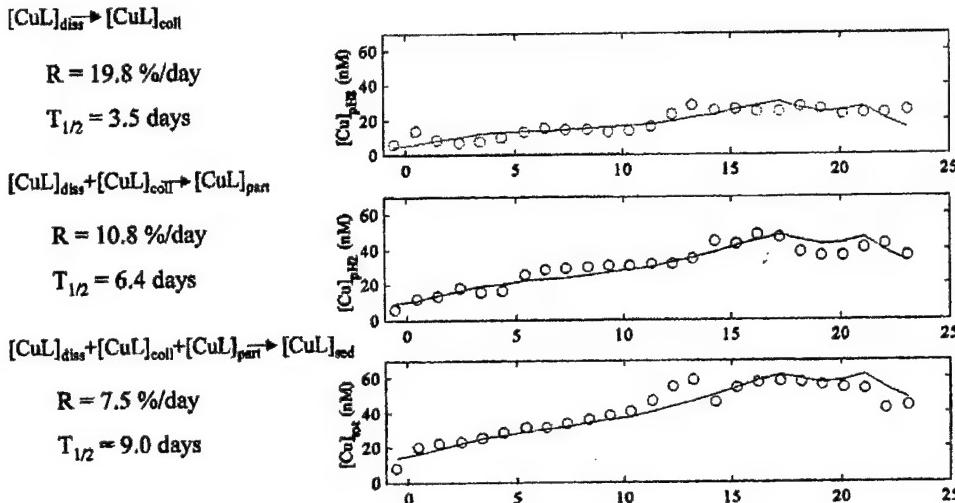
Figure 44. Results of species modeling. Line: model; circles: experimental points.

In conclusion, a one-dimensional model of San Diego Bay has been calibrated by using salinity. This model fits the experimental data satisfactorily. When applied to the total copper balance, the model provides the following indications: (1) Nonconservative processes are of equal or greater importance to conservative processes in controlling the fate of copper. Approximately 60% of the copper entering San Diego Bay probably ends up in the sediment. (2) Time scales of aggregation and sedimentation are comparable to overall bay residence times and exceed local time scales of flushing. (3) The model predicts free copper, viz. the copper activity, by assuming chemical equilibrium and a uniform stability constant for L. Thus, the model also predicts possible toxicity provided that data for L are available.

DETERMINATION OF CU LIGANDS BY VOLTAMMETRY (S. Skrabal)

Copper-binding ligands in natural waters may be determined in solution by titration with copper ion by using a number of sensors for the free ion ($Cu^{++}_{(aq)}$). These sensors may be potentiometric, spectrophotometric, or voltammetric—the only limitation being that the "readouts" be specific for the ion. Two types of voltammetry have seen wide use for this purpose: anodic stripping voltammetry (ASV) on the mercury-film glassy carbon, rotating-disk electrode (MFGC-RDE) and ligand-exchange, cathodic-stripping voltammetry (LE-CSV) on the hanging mercury drop electrode (HMDE). Both methods have their respective advantages and will be discussed below.

Anodic Stripping Voltammetry

The ASV technique is the most direct. A copper ion reducing potential is applied to the working electrode in a polarographic electrolysis cell connected to a 3-electrode potentiostat as shown in

59

Figure 45. The electrode is usually an RDE rotating at high velocity. This parameter limits the residence time of any Cu-complexes in the diffusion layer and favors measurement of the free ion.

To potentiostat

Pt elec.

Ref. elec.

Working elec.

- Usually performed with rotating glassy carbon disc-thin mercury-film electrode
- High-rotation rates (up to 5000 rpm)
- Minimal sample disturbance
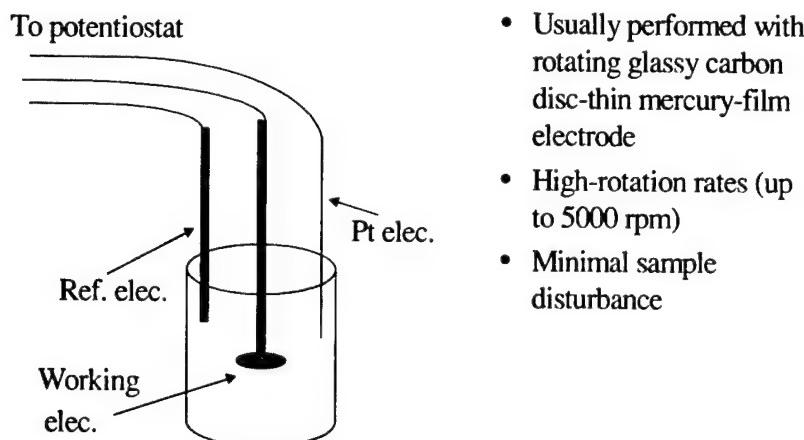
Figure 45. Typical voltammetric cell for ligand determination.

The potential applied to the working electrode is also set as to provide a minimum overvoltage over the polarographic reduction potential ($E_{1/2}$) of the copper ion in a seawater medium. The potential also serves to discriminate against organic copper complexes with relatively large formation constants but does not discriminate against "labile" organic or inorganic copper complexes. The titration is performed by making small additions of copper ion to the solution and by measuring the resulting Cu peak increase. Initial Cu additions are nondetectable because the Cu-organic ligand being formed is not detectable under the experimental conditions (Figure 46). The "break" in the plot from a low or no signal to a continually increasing signal indicates that the organic ligand (or class of ligands) has been saturated with Cu ion within the timeframe of the addition. The titration curve becomes linear when all ligands or ligand classes have been saturated.

NO COMPLEXATION

COMPLEXATION

- Cu titration performed
- Strongly complexed Cu species (CuL) not reduced, hence not detected.
- Absence of signal and curvature in plot indicate presence of strong complexing ligands
- Provides very good estimate of Cu' and of strong ligands with K'_{cond} $\approx 10^8 - 10^{12}$
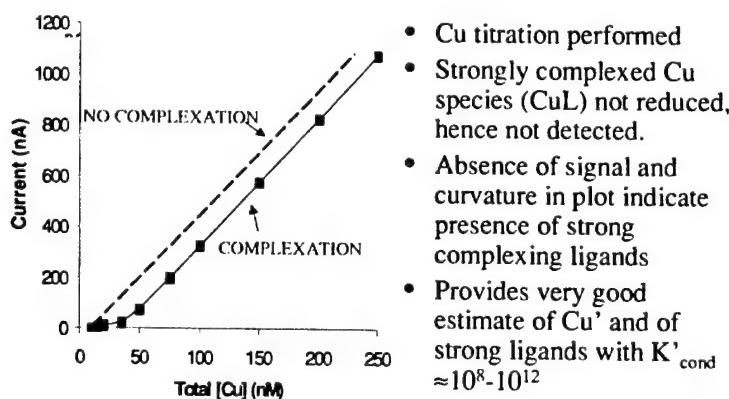
Figure 46. Cu titration of a hypothetical organic ligand in seawater using ASV.

Table 5 shows some of the results obtained using this technique to detect organic ligands in natural waters. However, in estuarine waters, rich in organic matter, there may be interferences on the RDE. In this case, use of an HMDE is preferred.

Table 5. Cu-complexes detected in Narrangassett and San Francisco Bays.

Kozelka and Bruland (1998) *Narragansett Bay*	$[L_1] = 16$ nM, $K'_{cond} = {}^3 10^{12.3}$ $[L_2] = 15$ nM; $K'_{cond} = 10^{10.5}$ $[L_3] = 57$ nM; $K'_{cond} = 10^{8.8}$
Donat (in Bruland et al., 2000) *Narragansett Bay*	$[L_1] = 13$ nM, $K'_{cond} = {}^3 10^{12.5}$ $[L_2] = 13$ nM; $K'_{cond} = 10^{10.1}$ $[L_3] = 46$ nM; $K'_{cond} = 10^{9.0}$
Donat et al. (1994) *S. San Francisco Bay*	$[L_2] = 60\text{-}80$ nM; $K'_{cond} = 10^{9.0}\text{--}10^{9.6}$

Competitive Ligand Equilibration Cathodic Stripping Voltammetry (CLE-CSV)

In this method, a thermodynamically well-characterized Cu-complexing ligand that is electroactive is added to a sample to "compete" with naturally occurring ligands. After a suitable deposition period to collect the newly formed Cu-(added) ligand complexes on the HMDE, a cathodic potential scan is applied to reduce Cu^{2+} in the complex to $Cu(0)$, and the resulting peak current is measured on an I vs. E plot. Depending on the strength and concentration of the metal-added ligand complex (MAL), different classes (of differing "strengths") of naturally occurring ligands can be characterized. Level of competition is defined by $\alpha = [MAL] / [M^{2+}]$, i.e., as α increases, the greater is the strength of the ligands detected. On the basis of titration results and equilibrium considerations, the metal speciation in the original sample can be calculated. The method yields estimates of $[L_x]$, K'_{cond}, $[Cu^{2+}]$. CSV does not distinguish between Cu^+ and Cu^{2+} (Leal & van den Berg, 1998). Several competing ligands have been used: 8-hydroxyquinoline ($\log \alpha = 5.0$ to 8.4), catechol ($\log \alpha = 4.0$ to 5.6), salicylaldoxime ($\log \alpha = 3.6$ to 5.8), benzoylacetone ($\log \alpha = 2.7$ to 4.1), and tropolone ($\log \alpha = 2.5$ to 4.5). The level of competition depends on ligand strength and concentration, and several competing ligands and strengths can be used to characterize ligand classes in a sample. The CLE-CSV method has been verified in an intercalibration exercise conducted in Narragansett Bay (Bruland et al., 2000). Essentially all (>99.97%) of the dissolved Cu was found to be complexed with organic ligands, resulting in "free" Cu^{2+} being approximately 10^{-13} M. A baseline study of copper complexation in the Cape Fear Estuary (North Carolina) showed that the concentration of complexing ligands exceeded the total copper concentration, thereby mitigating the toxicity of the copper ion (Figure 47).
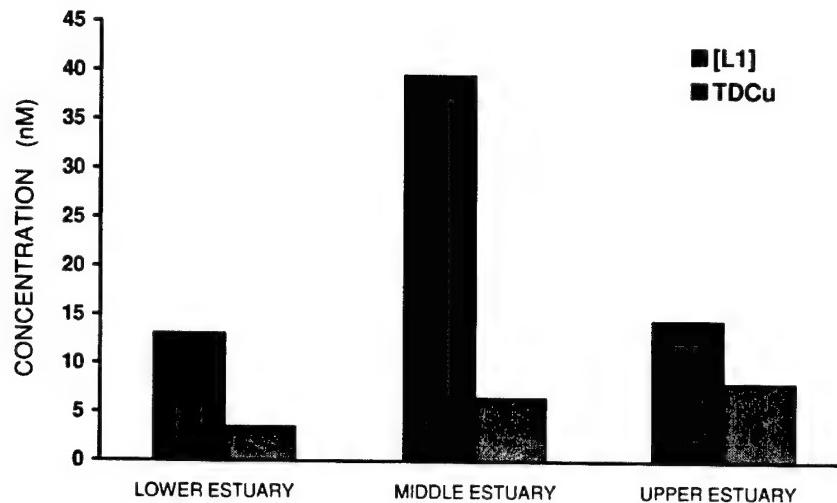
Figure 47. Total strong ligand and total copper in waters of the Cape Fear Estuary.

FLUORESCENCE-BASED BIOSENSING OF CU(II) IN SEAWATER (R. Thompson, H.-H. Zeng, M. Cramer, and C. A. Fierke)

For some time, we have been developing fluorescence-based metal ion biosensors with a view to applying them to analyzing ions such as Cu(II) in complex media such as seawater. To date, in the laboratory, we have demonstrated that these sensors respond reversibly to subpicomolar concentrations of free Cu(II) in less than 1 minute, and that these sensors permit its quantitation in metal-ion buffer systems, and that divalent ions such as calcium (Ca), magnesium (Mg), strontium (Sr), cobalt (Co), nickel (Ni), and cadmium (Cd) do not interfere at their ordinary concentrations.

The objective of the present study is to develop a real-time sensor for the Cu ion, with subparts-per-billion sensitivity in ship's waste-water discharge, where it should be able to determine pM Cu(II) reversibly in the presence of mM Ca(II) and Mg(II). A secondary requirement is that the sensor-package will function as a field instrument, viz., it should be rugged, relatively insensitive to large fluctuations in temperature, easy to calibrate, and have low power requirements.

We have based the sensor on human carbonic anhydrase II (CA II) from erythrocytes. While CA is a well-known and highly selective sensor for zinc (Zn), we have shown that other metals that bind to carbonic anhydrase induce proximity-dependent (fluorescent) quenching. Thus, Cu(II), Co(II), and NI(II) may be determined by changes in fluorescence intensity or, preferably, by changes in anisotropy or lifetime. An important advantage of CA-based sensors is the ability to modify the sensitivity and selectivity of the sensor by subtle changes to the protein structure. Figure 48 shows the selectivity and sensitivities of several variants of CA, together with that of a fluorescent indicator (Fura-2), in comparison to the total concentrations of the respective metals in seawater.
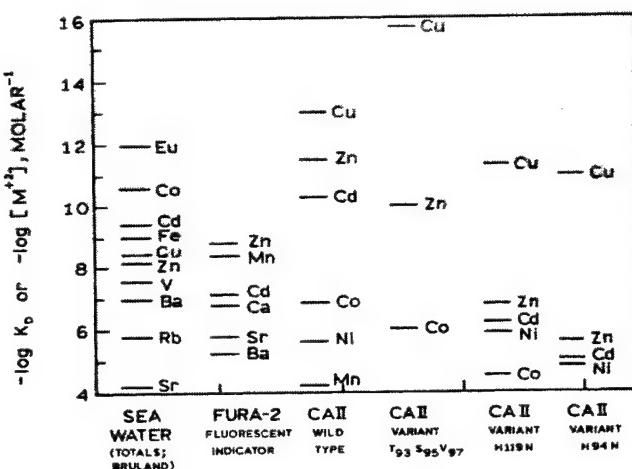
Figure 48. Selectivity of sensor transducers. Affinities for various metal ions are indicated by horizontal lines.

The selectivity of CA II for Cu over Zn may be enhanced by the ligands surrounding the metal binding site. For example, metal ligands H94, H119, and H96 form a tetrahedral binding site and are supported by a flexible beta-sheet, which favors the retention of Zn(II). By reducing the volume of hydrophobic amino acids at critical positions, the metal affinity and selectivity for Cu(II) can be improved from forty-fold over Zn(II) to one million-fold in the best example (Figure 49).
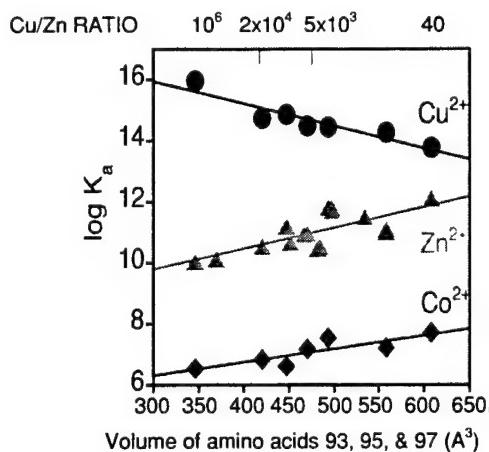
Figure 49. Sensitivity for Cu(II) is improved by reducing the volume of aromatic amino acids.

The fluorescence lifetime of metal-bound CA II and the resulting phase angles reflect the fractional saturation of binding sites with metal ion; the fluorescence lifetime and resulting phase angles, therefore, also reflect the metal-ion concentration in solution. This dependency is shown in Figure 50, which demonstrates how phase angle varies as a function of concentration in a buffer solution and in buffered artificial seawater.
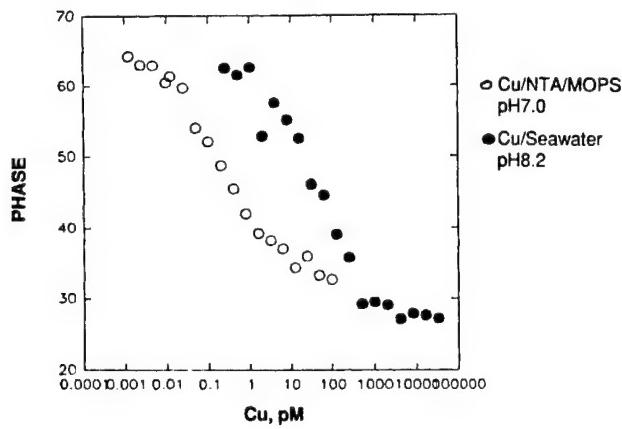
Figure 50. Conditional stability constant in buffer
and model seawater.

Detection systems made with immobilized CA respond rapidly to added copper concentrations in seawater at sub-ppb levels, responding to nanomolar additions in less than 1 minute and exhibiting a reversible response (Figure 51).
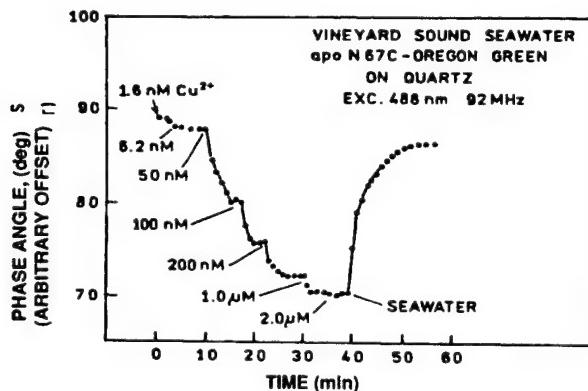
Figure 51. Immobilized CA detects ppb Cu^{2+} levels
fast in seawater.

The selectivity of CA II for the Cu(II) ion, rather than organically complexed copper, has permitted speciation analysis at levels comparable to those achievable by voltammetry or potentiometry. Transducing the Cu(II) binding as a change in fluorescence anisotropy (polarization) offers comparable sensitivity and speed of response in a steady-state instrument, which is much cheaper than a lifetime instrument. Heretofore, fluorescence anisotropy has been widely applied to fluorescence polarization immunoassay. Fluorescence anisotropy gives a very robust signal, free from artifact, similar to ratiometric techniques. When applied to the detection of Cu(II) in seawater, fluorescence anistropy provides picomolar sensitivity (Figure 52).

64
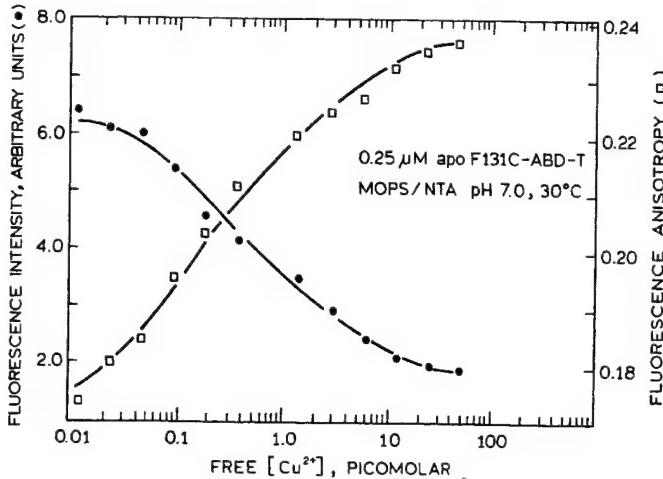
Figure 52. Anisotropy measures sub-ppb Cu.

Issues and future plans include the incorporation of new and more robust variants into the sensor, immobilization of the CA II for anisotropy-based sensing, and the use of Commercial-off-the-Shelf (COTS) instrumentation for field measurements. We also plan to immobilize the protein at the end of an optical fiber to measure oceanic Cu(II) levels in situ. Furthermore, we intend to expand this technique to the measurement of other analytes such as lead (Pb), mercury (Hg), cadmium (Cd), and iron (Fe). We also intend to explore the application of this technique beyond the environmental field, into neuroscience and other clinical applications.

ACTIVITY MEASUREMENTS WITH THE CU-ION-SELECTIVE ELECTRODE (R. De Marco)

The Cu-ion-selective electrode (ISE) determines the activity of Cu^{2+} —a master variable in the regulation of Cu uptake and toxicity. The Cu ISE is inexpensive, simple to use, and suitable for ex-situ and in-situ field analyses. The Cu ISE is also amenable to remote and/or rapid throughput automated analyses (e.g., satellite datalogging, flow analysis, etc.). The electrode is prepared by precipitation out of Ag_2S and CuS and is commercially available. After introduction by Orion in the early 1970s, the Cu ISE found immediate use in the study of freshwaters. However, problems were encountered when the ISE was applied to the measurement of Cu in seawater. Even though the Cu ISE showed remarkable sensitivity to nanomolar Cu^{++} increases in seawater, the response could be greater than the theoretical Nernstian response of 30 mv/decade change in concentration. For example, Westall et al. (1979) and Lanza (1979) showed a chloride interference leading to super-Nernstian response at very high Cu activities (> 10^{-5} M), viz.

$$Cu^{2+}_{(aq)} + CuS_{(s)} \longrightarrow 2Cu+_{(aq)} + S_{(s)}$$

$$Cu^{+}_{(aq)} + nCl^{-}_{(aq)} \longrightarrow CuCln_{(2-n(aq))}$$
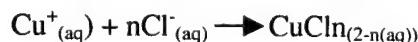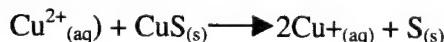
at [Cu^{2+}] of 10^{-7} to 10^{-4} M, and at higher levels

$$Ag_2S_{(s)} + Cu^{2+}_{(aq)} + 2Cl^{-}_{(aq)} \longrightarrow CuS_{(s)} + 2AgCl_{(s)}$$

65
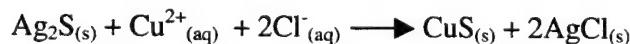
Despite the fact that Cu^{++} in seawater is seldom greater than 5×10^{-8} M, the super-Nernstian response caused seawater investigators to abandon use of the electrode for nearly 20 years. It was not until 1993 that it was shown that the electrode operated properly at seawater concentrations, and that the "chloride interference" observed at high concentrations did not occur at low copper concentrations because it was kinetically hindered (De Marco et al., 1998). Similarly, in the same year it was also shown that the anomalous response was due to the presence of Lewis bases in seawater, probably of organic origin (Belli and Zirino 1993). Nevertheless, difficulties with the use of the electrode remained. The electrode itself was sensitive to light, which, in the presence of oxygen, caused AgCl to form at its surface (De Marco, 1994), thereby turning the electrode into a Cl⁻ sensor; copper ion was released by the crystal sensing surface, thereby contaminating the diffusion layer and ultimately significantly affecting the copper concentration in solution. Finally, dissolved organic matter adsorbed on the electrode might appreciably affect the potential. These effects were studied by De Marco (1996) and Zirino et al. (1998a), who determined that the electrode should be kept out of direct sun-light and either be rotated or used with flowing solutions (to minimize the electrode's contribution to Cu^{++} in the diffusion layer) (Zirino et al., 2002) and that dissolved organic matter did not significantly affect its potential (De Marco et al., 1998).

Although, at present, no other sensor exists that directly measures Cu activity in seawater, Cu-ISE values in organic-free seawater at pH 2 have yielded values within experimental error of total copper values, measured with graphite furnace atomic absorption spectroscopy (GFAAS), when the total concentration was corrected for complexing effects (Mackey et al., 1996). A recent Cu-ISE application to the analysis of estuarine samples at pH 2 resulted in the development of a continuous flow technique that yielded ISE total values within 0.1 to 0.3 pCu units of GFAAS determinations (Eriksen et al., 1999). At pH 8, or at the natural pH of seawater for which there are no standards, Cu-ISE measurements have consistently yielded pCu values comparable to literature values when using indirect techniques. In bioassays, pCu values measured directly have correlated comparably to LC50 values in the literature, thereby firmly establishing a relationship between the direct activity measurement and biological effects (Rivera-Duarte et al., 2002).

Overall, the Cu-ISE has been embraced by the scientific community as a useful tool in studies of Cu speciation. In seawater, recent studies conducted in San Diego Bay, California, and in McQuarrie Harbor, Tasmania, copper species were measured using the ISE, voltammetry, and GFAAS. The data for total and free copper were interpreted according to the following equilibrium:

$$Cu_{(aq)} + L_{(aq)} \longrightarrow CuL_{(aq)}; \qquad \beta = CuL_{(aq)}/\{Cu_{(aq)} \times L_{(aq)}\}$$
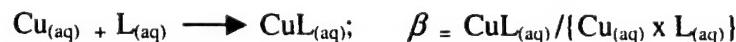
yielding comparable equilibrium constants to those expected for natural ligands (Zirino et al., 1998; Eriksen et al., 2000). A comprehensive literature search reveals over 40 applications of the Cu ISE since the early 1990s in fields as broad as marine chemistry, biochemistry, toxicology, wine chemistry, soil science, and geochemistry (De Marco, in prep.). In the future, there are several new and exciting possibilities of using ISEs to monitor chemical processes in individual particles or cells by using a microelectrodes and/or ion-selective electrode–atomic force microscopy (ISE–AFM).

USER AND REGULATORY GROUP WRAP-UP SESSION AND RECOMMENDATIONS

(Facilitator: P. F. Seligman)

After the general session, a discussion was held among Navy users and regulators to define issues of concern, technology gaps, and how, from the user perspective, science can help resolve some of these issues.

COPPER DISCHARGES OF CONCERN

Generally, the Navy Cu discharges discussed in the first workshop remain the same. Typically, the leading source of copper in Navy harbors is from hull leachates off civilian and military copper-based paints. However, recent ONR studies, have shown that the release rate of copper from Navy hulls is much lower than previously estimated—probably because of environmental factors such as the presence of biofilm. Since copper has a lower release-rate value, other ship discharges such as cooling water, gray water, firemain water, etc. will represent a higher percentage of the total Cu loading in Navy harbors. It is still likely, however, that hull leachates from civilian and Navy hulls will be the predominate source of copper in many waterbodies. Other sources include hull husbandry from in-water hull cleaning and drydock operations. Drydocks are a particular Navy concern because of Notices of Violation (NOVs) issued for Cu discharges either from paining/paint removal operations or vessel dismantling/hull recycling operations, particularly with submarines. Another source of copper from drydocks is hull-painting preparation with high-pressure water (2000 psi) and paint removal with high-pressure water (20,000 psi). This process creates a large amount of liquid waste that has to be treated and may result in copper exceedances.

Stormwater (nonpoint source [NPS]) was discussed as a growing issue and is often a significant source of copper, particularly in areas with substantial rainfall and with large watersheds in urban areas. Of particular note were new requirements to evaluate NPS by using whole effluent toxicity (WET) testing, which is likely to result in violations. Finally, naval and other Industrial Wastewater Treatment Plant (IWTP) effluents, particularly from metal finishing, have received NOVs for exceeding copper limits.

REGULATORY ISSUES

Uniform National Discharge Standards (UNDS) are being developed jointly by the DoD and the U.S. EPA and continue to be the driving force in discharge regulations and pollution control for all vessels of the armed forces. Many of the discharges defined as requiring Marine Pollution Control Devices (MPCDs) have copper as one of the contaminants. MPCDs being considered to reduce Cu inputs include developing new robotic in-water hull-cleaning devices with post treatment of wastes and new AF coatings with no, or reduced, Cu biocide.

There is a growing awareness of the need to address sediment contamination. A few states have developed sediment standards (e.g., Washington and Florida). The U.S. EPA is considering sediment criteria. Regulations vary depending on whether the contamination is related to a cleanup action under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) or part of maintenance dredging under the Marine Protection Research and Sanctuaries Act (MPRSA) and the Clean Water Act (CWA). California is beginning to manage sediments under its clean water law through the toxic hotspot program, and copper is one of the common contaminants of concern.

The CWA, section 303(d) is beginning to regulate mass loading of Cu and other contaminants through the use of Total Maximum Daily Load (TMDL)/ Waste Load Allocation (WLA) provisions. This regulation will definitely impact facility discharges (e.g., in shipyards) shipyards. However, the Uniform National Discharge Standards will probably not be directly impacted by the TMDL process. Nevertheless, ship discharges may be used in calculating total loading into estuaries and harbors.

A number of questions and issues were raised concerning discharge requirements and regulations:

- What is the appropriate measure of compliance? Total? Dissolved? or possibly in the future, copper ion activity? Even though the current U.S. EPA WQC are defined in terms of dissolved Cu, many permits still require measurements as total recoverable Cu, which occasionally results in NOVs.

- How are we going to bring the bioavailability of Cu into copper criteria and standards?

- Where should the point of compliance be?—at the end of the pipe, using a dilution zone, toxicity testing, or some combination?

- How can we move away from using end-of-pipe concentrations and move toward total loading watershed loading?

- How can we best apply (and scientifically justify) background concentrations in determining discharge limits. In some cases, regulators are mandating discharge limits less than background water levels, which are virtually unachievable.

- The U.S. EPA has listed copper on the Level II Persistent, Bioaccumulative, and Toxic Chemical Program. Does it make sense to be listed?

TECHNOLOGY GAPS

The user/regulatory group felt there was a need to better understand copper loading and to develop improved, scientifically sound approaches to regulating copper. The group suggested the following technologies were needed:

Water Column

- Improved real-time sensors for copper

- Sensors to detect fouling to enhance noncontaminating cleaning procedures

- Improved modeling and verification of Cu fate and effects and a release rate model for improved prediction of environmental loading

Sediments

- Ligand flux, resuspension, and in-situ detection capability

AF Coatings

- Standardized release-rate measurement technology that provides realistic loading estimates and is cost effective

Pollution Control

- An effective and truly nontoxic fouling control coating system (long-term goal)

- Improved and low-cost metal removal from waste streams (e.g., effluents from in-water hull-cleaning effluents)

- Piping systems with no, or reduced, Cu discharge

- Improved management of stormwater runoff

HOW CAN SCIENCE HELP

The following suggestions were made by the user group to define and resolve some of the outstanding science gaps:

- Define clearly how ligands (e.g., L1 and L2) and complexation capacity relate to WQC and copper regulation in general

- Make better linkage of discharge concentration/loading to receiving water impacts (i.e., what is the carrying capacity of the water and how do you calculate it?)

- Define acceptable (both scientifically and regulatory) method(s) for measuring Cu ion activity

- Make a better linkage between Cu water loading and Cu sediment

WORKSHOP SCIENTIFIC RECOMMENDATIONS

(Facilitator: A. Zirino)

A meeting of the scientific group took place after the presentations. Several areas were identified as important to the full understanding of the issues that affect copper and its toxicity in the marine environment. However, in terms of a general understanding, it was clear that a consensus existed among the scientists on the principal issue: copper complexation with organic matter was real and ubiquitous. In every case presented at the workshop and in virtually every recent article in the literature, copper was complexed to a very high degree (>90 to 99%) with (presumably) organic ligands, which may be either dissolved, colloidal, or particulate in nature. Complexation, which, in this case, may include copper directly adsorbed on colloidal matter, or copper complexed with organic matter and then incorporated into a colloidal or particulate, largely organic matrix, rendered copper less labile and potentially less available to organisms than copper as the $Cu(II)_{aq}$ ion.

In light of the above and the observation that $Cu(II)_{aq}$ is the best measure of potential toxicity (Campbell, 1995), the regulatory dilemma of "free" copper vs. "total (dissolved) copper" is exposed once again. On the other hand, this dilemma now is a testable hypothesis. Listed below are questions that may be asked (and tested).

1. ARE "TOTAL (DISSOLVED)" COPPER AND "FREE" COPPER INDEPENDENT VARIABLES IN THE ENVIRONMENT?

Presently, the answer appears to be "yes" and "no," although the database is very limited. Zirino et al. (1998b), in an early study of San Diego Bay, hedged on this because they found a direct correlation between the two copper entities in Shelter Island Inlet, and no correlation in the center of the bay. These results have now been confirmed by recent work in San Diego Bay (Chadwick et al., this work), which shows a persistent inverse correlation between total copper and free copper at the center and shallow southern portions of the Bay. This inverse correlation has been observed regularly over 2 years of sampling.

So, is this a general relationship? What about other bays and harbors that may experience more pronounced climatic variations? Will there be spatial differences within the estuaries? If indeed a linear relationship exists, will the ratio between free and total copper be a constant, or will it exhibit temporal and spatial variability? Clearly, more data from other estuarine environments are needed. This last question also brings us to the next issue.

2. WHAT IS THE ROLE OF ORGANIC MATTER IN AMELIORATING THE ENVIRONMENT?

This question strikes at fundamental principles that govern the marine ecosystem. What is the source of the material that binds copper and renders it less toxic? Laboratory and field experiments indicate that this material is produced and modified by microorganisms (plankton and bacterio-plankton) in response to copper stress. "What we don't know about them includes in-situ sources and sinks, the molecular structures, the mechanisms of actual complexation, and the actual physiological mechanisms of production. It is now established that various types of bacteria in culture excrete ligands with copper binding characteristics similar to the L_1 ligands that are present in seawater" (Gordon, this work).

Once again, more data are needed about temporal and spatial variability as well as geographic and climatic variability. Nevertheless, Cu-complexation appears to be ubiquitous—an indispensable

survival tool for microorganisms, and, as such, may indeed be predictable, if other aspects of the environment (light, nutrients, species composition, etc.,) are known.

3. CAN CU(II)$_{aq}$ BE MEASURED ACCURATELY IN SEAWATER?

If Cu(II)$_{aq}$ indeed proves to be an independent variable, will it be possible to measure it easily and accurately? This attribute is indispensable if water quality standards based on Cu(II)$_{aq}$ are to be adopted. Ion-elective electrodes appear promising, as well as any other sensitive technique that presumes equilibrium between the sensor and the analyte. (Voltammetry-based systems cannot measure Cu(II)$_{aq}$ directly, although a limiting value of Cu(II)$_{aq}$ may be computed from voltammetric currents.) Formerly, interferences have diminished enthusiasm for the use of the jalpaite-type ISE in seawater; however, a better understanding of this sensor has been obtained, and the jalpaite-type ISE functions remarkably well in flowing systems (Eriksen et al., 2001; De Marco, this work). Also, a chalcogenide glass-based Cu(II) ISE has been developed recently and appears to suffer from far fewer interferences than the jalpaite-based ISE. Fluorescence anisotrophy based on carbon anhydrase (Thompson et al., this work) also appears to be a very promising and interference-free copper ion detector. Nevertheless, no copper ion sensor has ever been calibrated against a certified Cu(II) standard because none exist for seawater. It is recommended that more development work be done in this area to reconcile and render accurate all of the many types of measurements of Cu(II)$_{aq}$ now available.

4. WHAT OTHER RECOMMENDATIONS CAN BE MADE?

a. Community effects. To truly assess the environmental toxicity of copper, it is important to determine how effects on individual species impact entire communities. Given the complexities of the natural environment, this endeavor promises to be a long and arduous project. Nevertheless, the known toxic effect of copper in nature is essentially on the phytoplankton/bacterioplankton level, and we can take comfort in the fact that the toxicity of copper is quite universally mitigated by the production of organic matter. Generally, the concentration of the copper-complexation capacity is within a factor of 2 of the total copper concentration. This fact points to a robust and possibly predictable control system at the microorganism level. Higher trophic levels may not respond to copper ion but may respond to other forms of copper. These would then require further study.

b. Release and complexation of copper at interfaces. It has been suggested that it would be useful to study the complexation mechanism of copper as it is released from ship hulls painted with copper-based anti-fouling paints. Indeed, if it could be shown that copper is released into the water as a complex, then its toxicity would already have diminished. This suggestion ignores the bacterially mediated transformations that may occur once the copper-complex enters the water column, but it points out that very little is known about processes at interfaces occurring in marine waters and that this is a promising area for further study.

CONCLUSIONS

1. The Navy user community and regulatory agencies are working hand-in-hand in a continuing effort to limit the impact of copper release on estuarine environments. The Navy effort is centered on developing new technology for the removal of copper from effluents and toward developing low-copper-release or copper-free hull coatings. However, as copper water-quality standards and permit limits are lowered, it is becoming increasingly difficult and cost prohibitive for Navy facilities to achieve regulatory compliance. Indeed, it is not even particularly clear how much effort is really needed since the present approach largely ignores the environment's own defenses against copper toxicity.

2. Natural mechanisms that mitigate copper toxicity in estuaries include dispersion and flushing, sedimentation and complexation. In the scientific literature, there is overwhelming evidence that at least 99.9% of dissolved copper is either complexed or otherwise associated with large dissolved or colloidal materials, principally of organic origin. Many of these organic materials are produced as a defense mechanism by the resident phytoplankton and modified by the bacterioplankton, in response to copper loading. Association with colloidal macromalecules leads to dispersion, sedimentation, and removal of copper from the water column.

3. The mitigating effects of complexation are being implicitly accepted by regulatory agencies through the use of water-effects-ratios (WERs) and through the endorsement of the Biotic Ligand Model (BLM).

4. While it is generally accepted that the copper fraction that is the best indicator of overall environmental toxicity is the available (noncomplexed) copper—the "free" copper activity is also the most thermodynamically sound quantity—this quantity is NOT being routinely measured at present nor is it being used in water-quality assessments, even though measurement techniques for it are available.

5. The reluctance of the regulatory agencies to endorse the direct measurement of copper activity (the biologically available copper) is hampering progress in several ways: (1) it provides no impetus to develop water-quality standards based on copper activity, even though they may be more scientifically relevant than standards based on concentrations, particularly at low concentration levels; (2) it provides no impetus to develop improved or alternate methods to measure copper activity; and (3) it does not encourage calibration, standardization, or the improvement of the techniques already available.

6. The result of the above is that, at present, and at the low copper levels found in estuaries, there are no reliable, scientifically sound, and realistic toxicity criteria on which to base estimates for future investments in Cu remediation technology. This suggests continually rising, and possibly unnecessary, costs for environmental protection in the future.

7. The intervening years between the First and Second ONR Copper Workshops have seen the realization of some of the recommendations made in the First Workshop, namely the integration of organic complexation of copper into hydrodynamic models, a further exploration into the role of organic matter in ameliorating toxicity, and the development and use of new techniques for estimating and measuring copper activity. The original issue of copper activity vs. copper concentration is no longer much of an issue. However, the development of a new toxicity scale based on activity will require a long-term, concerted, interdisciplinary scientific effort, perhaps similar to the effort that went into developing copper concentration standards. Copper activity measurement protocols will

have to be optimized and standardized, and then they will need to be applied to a large number of organisms and conditions. This process would be a long-term investment, to be sure, but it would be more environmentally relevant and cost efficient when compared to the scientifically dubious, concentration-based approach used presently.

REFERENCES

Belli. S. L. and A. Zirino. 1993. "Behavior and Calibration of the Cu(II) Ion Selective Electrode in High Chloride Media and Marine Waters," *Analytical Chemistry*, vol. 65, pp. 2583–2589.

Bruland, K. W., E. L. Rue, J. R. Donat, S. A. Skrabal, and J. W. Moffett. 2000. "Intercomparison of Voltammetric Techniques to Determine the Chemical Speciation of Dissolved Copper in a Coastal Seawater Sample," *Analytica Chimica Acta,* vol. 405, pp. 99–113.

Bury, N., F. Galvez, and C. Wood. 1999. "Effect of Chloride, Calcium, and Dissolved Organic Carbon on Silver Toxicity: Comparison Between Rainbow Trout and Fathead Minnows," *Environmental Toxicology and Chemistry*, vol. 18, pp. 56–62.

Campbell, P. G. C., 1995. "A Critique of the Free Ion Activity Model," *Metal Speciation and Bioavailability in Aquatic Systems*, (A. Tessier and D. R. Turner, eds.), John Wiley and Sons, Chichester.

Chadwick, D. B., C. N. Katz, and J. L. Largier. 1995. "Contaminant Transport Measurements in San Diego Bay," *Proceedings of Ooceans '95*, San Diego, CA, vol. 3, pp. 1691–1699.

De Marco, R. 1994. "Response of Cu(II) Ion-Selective Electrodes," *Analytical Chemistry*, vol. 66, pp. 3202–3207.

De Marco, R. 1996. "Surface Studies of the Jalpaite-Based Copper(II) Ion-Selective Electrode Membrane in Seawater," *Marine Chemistry*, vol. 55, pp. 389–398.

De Marco, R., R. Eriksen, and A. Zirino. 1998. "Electrochemical Impedance Spectroscopy Study of the Response Mechanism of the Jalpaite Cu(II) Ion-Selective Electrode in Seawater," *Analytical Chemistry*, vol. 70, pp. 4683–4689.

De Marco, R. In prep.

De Schamphelaere, K.A.C. and C. R. Janssen. 2002. "A Biotic Ligand Model Predicting Acute Copper Toxicity for Daphnia Magna: the Effects of Calcium, Magnesium, Sodium, Potassium and pH," *Environmental Science and Technology*, vol. 36, pp. 48–54.

De Schamphelaere, K.A.C., D. G. Heijerick, and C. R. Janssen. 2002. "Refinement and Field Validation of a Biotic Ligand Model Predicting Acute Copper Toxicity to *Daphnia magna*," submitted to *Comparative Biochemistry and Physiology*, special BLM-Issue.

Di Toro, D. M., H. E. Allen, H. L. Bergman, J. S. Meyer, P. R. Paquin and R. C. Santore. 1999. "A Biotic Ligand Model of the Acute Toxicity of Metals. I. Technical Basis," Section 3 in Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments," report to the U.S. EPA Science Advisory Board, Office of Water, Office of Research and Development, Washington, DC, USEPA-822-E-99-001, pp. 3-1 to 3-26.

Di Toro, D. M., H. E. Allen, H. L. Bergman, J. S. Meyer, P. R. Paquin, and R. C. Santore. 2000. "The Biotic Ligand Model: A Computational Approach for Assessing the Ecological Effects of Metals in Aquatic Systems," published by the International Copper Association, Ltd., Environmental Program as part of its series on "Copper in the Environment and Health."

Di Toro, D. M., H. E. Allen, H. L. Bergman, J. S. Meyer, P. R. Paquin, and R. C. Santore. 2001. "A Biotic Ligand Model of the Acute Toxicity of Metals. I. Technical Basis," *Environmental Toxicology and Chemistry*, vol. 20, no. 10, pp. 2383–2396.

Donat, J. R., K. A. Lao, and K. W. Bruland. 1994. "Speciation of Dissolved Copper and Nickel in South San Francisco Bay: a Multi-Method Approach," *Marine Chemistry*, vol. 36, pp. 329–363.

Erickson, R. J., D. A. Benoit, V. R. Matson, H. P. Nelson, Jr., and E. N. Leonard. 1996. "The Effects of Water Chemistry on the Toxicity of Copper to Fathead Minnows," *Environmental Toxicology and Chemistry*, vol. 15, pp. 181–193.

Eriksen, R. S., D. J. Mackey, P. Alexander, R. De Marco, and X. D. Wang. 1999. "Continuous Flow Method for Evaluating the Response of a Copper Ion Selective Electrode to Total and Free Copper in Seawater," *Journal of Environmental Monitering*, vol. 1, pp. 483–488.

Eriksen, R. S., D. J. Mackey, R. van Dam, and B. Nowak. 2001. "Copper Speciation and Toxicity in Macquarie Harbour, Tasmania: an Investigation Using a Copper Ion Selective Electrode," *Marine Chemistry*, vol. 74, pp. 99–113.

Galvez, F. and C. M. Wood. 1997. "The Relative Importance of Water Hardness and Chloride Levels in Modifying the Acute Toxicity of Silver to Rainbow Trout (*Oncorhynchus mykiss*)," *Environmental Toxicology and Chemistry*, vol. 16, no. 11, pp. 2363–2368.

Grosell, M., C. Hogstrand, C. M. Wood, and H. J. M. Hansen. 2000. "A Nose-to-Nose Comparison of the Physiological Effects of Exposure to Ionic Silver Versus Silver Chloride in the European eel (*Anguilla anguilla*) and the Rainbow Trout (*Oncorhynchus mykiss*)," *Aquatic Toxicology*, vol. 48, pp. 327–342.

Guo, L., L.-S. Wen, D. Tang, and P. H. Santschi. 2000a. "Re-Examination of Cross-Flow Ultrafiltration for Sampling Marine Colloids: Evidence from Molecular Probes," *Marine Chemistry*, vol. 69, no. 1-2, pp. 75–90.

Guo, L., P. H., Santschi, and K. W. Warnken. 2000b. "Trace Metal Composition of Colloidal Material in Estuarine and Marine Environments," *Marine Chemistry*, vol. 70, pp. 257–275.

Guo, L., B. Hunt, and P. H. Santschi. 2001. "Ultrafiltration Behavior of Major Ions (Na, Ca, Mg, F, Cl, and SO4) in Natural Water," *Water Research*, vol. 35, no. 6, 1500–1508.

Heijerick, D. G., K. A. C. De Schamphelaere, and C. R. Janssen. 2002. "Predicting Acute Zinc Toxicity for Daphnia Magna as a Function of Key Water Chemistry Characteristics: Development and Validation of a Biotic Ligand Model," accepted in *Environmental Toxicology and Chemistry*.

Hogstrand, C. and C. M. Wood. 1998. "Towards a Better Understanding of the Bioavailability, Physiology, and Toxicity of Silver in Fish: Implications for Water Quality Criteria," *Environmental Toxicology and Chemistry*, vol. 17, pp. 547–561.

Hung, C.-C. and P. H. Santschi. 2001. "Spectrophotometric Determination of Total Uronic Acids in Seawater Using Cation Exchange Separation and Pre-Concentration by Lyophilization. *Analytica Chimica Acta*, vol. 427, pp. 111–117.

Hung, C.-C., D. Tang, K. Warnken, and P. H. Santschi. 2001. "Distributions of Carbohydrates, Including Uronic Acids, in Estuarine Waters of Galveston Bay," *Marine Chemistry*, vol. 73, pp. 305–318.

Johnson, H. D., J. G. Grovhoug, and A. O. Valkirs. 1998. "Copper Loading to U.S. Navy Harbors: Norfolk, VA; Pearl Harbor, HI; and San Diego, CA," TD 3052, SSC San Diego, San Diego, CA, p. 58.

Katz, C. N. 1998. "Seawater Polynuclear Aromatic Hydrocarbons and Copper in San Diego Bay," TR 1768, SSC San Diego, San Diego, CA, p. 32.

Kozelka, P. B. and K. W. Bruland, 1998. "Chemical Speciation of Dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island," *Marine Chemistry*, vol. 60, pp. 267–282.

Lanza, P. 1979. *Analytica Chimica Acta,* vol. 105, pp. 53–56.

Largier, J. L., J. T. Hollibaugh, and S. V. Smith. 1997. "Seasonally Hypersaline Estuaries in Mediterranean-climate Regions," *Estuarine Coastal and Shelf Science*, vol. 45, pp. 789–797.

Leal, M. F. C. and C. M. G. van den Berg. 1998. "Evidence of Strong Copper (I) Complexation by Organic Ligands In Seawater," *Aquatic Geochemistry*, vol. 4, pp. 49–75.

Lewis, S. D. and W. M. Lewis. 1971. "The Effect of Zinc and Copper on the Osmolality of Blood Serum of the Channel Catfish *Ictalurus punctatis Rafinesque* and Golden Shiner *Notemigonus crysoleucas Mitchell*," *Transactions of American Fisheries Society*, vol. 100, pp. 639–643.

Lieberman, S. H., V. Homer, and P. F. Seligman. 1985. "In-situ Determination of Butyltin Release Rates from Antifouling Coating on Navy Test Ships," TR 1027, Naval Ocean Systems Center,[*] San Diego, CA, p. 17.

Mackey, D. J. and A. Zirino. 1994. "Comments on Trace Element Speciation, or Do Onions Grow in the Sea?" *Analytica Chimica Acta*, vol. 284, pp. 635–647.

Mackey, D. J., E. C. V. Butler, P. D. Carpernter, H. W. Higgins, J. E. O'Sullivan, and R. B. Plaschke. 1996. "Trace Elements and Organic Matter in a Pristine Environment: Bathurst Harbor, Southwestern Tasmania," *Science of the Total Environment*, vol. 191, pp. 137–151.

Mantel, L. H. and L. L. Farmer. 1983. "Osmotic and Ionic Regulation," Chapter 2 in *Internal Anatomy and Physiological Regulation*, vol. 5, of *The Biology of Crustacea*, Academic Press, (L.H. Mantel, ed.), pp. 53–161.

McDonald, D. G., H. Hobe, and C. M. Wood. 1980. "The Influence of Calcium on the Physiological Responses on the Rainbow Trout (*Salmo gairdneri*) to Low Environmental pH," *Journal of Experimental Biology*, vol. 88, pp. 109–131.

McGeer, J. C. and C. M. Wood. 1998. "Protective Effects of Water Cl⁻ on Physiological Responses to Waterborne Silver in Rainbow Trout," *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 65, pp. 2447–2454.

McGeer, J. C., R. C. Playle, C. M. Wood, and F. Galvez. 2000. "A Physiologically Based Biotic Ligand Model for Predicting the Acute Toxicity of Waterborne Silver to Rainbow Trout in Freshwaters," *Environmental Science and Technology*, vol. 34, pp. 4199–4207.

Morgan, I. J., R. P. Henry, and C. M. Wood. 1997. "The Mechanism of Acute Silver Nitrate Toxicity in Freshwater Rainbow Trout (*Oncorhynchus mykiss*) is Inhibition of Gill Na+ and Cl- Transport," *Aquatic Toxicology*, vol. 38, pp. 145–163.

---

[*] Now SSC San Diego

Packer, R. K. and W. A. Dunson. 1970. "Effects of Low Environmental pH on Blood pH and Sodium Balance of Brook Trout," *Journal of Experimental Zoology*, vol. 174, pp. 65–72.

Pagenkopf, G. 1983. "Gill Surface Interaction Model for Trace Metal Toxicity to Fishes: Role of Complexation, pH, and Water Hardness," *Environmental Science and Technology*, vol. 17, pp. 342–347.

Pagenkopf, G., R. Russo, R. Thurston. 1974. "Effect of Complexation on Toxicity of Cu to Fishes," *Journal of the Fisheries Research Board of Canada*, vol. 31, pp. 462–465.

Paquin, P. R., D. M. Di Toro, R. C. Santore, D. Trivedi, and K. B. Wu. 1999. "A Biotic Ligand Model of the Acute Toxicity of Metals. III. Application to Fish and Daphnia Exposure to Silver," Section 3 in *Integrated Approach to Assessing the Bioavailability and Toxicity of Metals in Surface Waters and Sediments*, a submission to the U.S. EPA Science Advisory Board, Office of Water, Office of Research and Development, Washington, DC, USEPA-822-E-99-001, pp. 3-59 to 3-102.

Paquin, P. R., V. Zoltay, R. P. Winfield, K. B. Wu, R. Mathew, R. Santore, and D.M. Di Toro. 2002. "Extension of the Biotic Ligand Model of Acute Toxicity to a Physiologically-based Model of the Survival Time of Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Silver," submitted to *Comparative Biochemistry and Physiology*.

Playle, R. C., D. G. Dixon, and K. Burnison, 1993. "Copper and Cadmium Binding to Fish Gills: Estimates of Metsl-Gill Stability Constants and Modeling of Metal Accumulation," *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 50, pp. 2678–2687.

Quigley, M. S., P. H. Santschi, L. Guo, and B. D. Honeyman. 2001. "Sorption Irreversibility and Coagulation Behavior of [234]Th with Surface-Active Marine Organic Matter," *Marine Chemistry*, vol. 76, pp. 27–45.

Quigley, M. S., P. H. Santschi, C.-C. Hung, L. Guo, and B. D. Honeyman. 2002. "Importance of Polysaccharides for [234]Th Complexation to Marine Organic Matter." *Limnology and Oceanography*, in press.

Rivera–Duarte, I. et al., 2002. In prep.

Santore, R. C., D. M. Di Toro, P. R. Paquin, H. E. Allen, and J. S. Meyer. 2001. "A Biotic Ligand Model of the Acute Toxicity of Metals. II. Application to Acute Copper Toxicity in Freshwater Fish and Daphnia," *Environmental Toxicology and Chemistry*, vol. 20, no. 10, pp. 2397–2402.

Santore, R., R. Mathew, P. R. Paquin, and D. M. Di Toro. 2002. "Development of a Biotic Ligand Model of Acute Toxicity for Zinc," submitted to *Comparative Biochemistry and Physiology*.

Santschi, P. H., E. Baluois, K. Wilkinson, J. Zhang, J. Buffle, and L. Guo. 1998. "Fibrillar Polysacchlorides in Marine Molecular Organic Matter as Imaged by Atomic Force Microscopy and Transmission Electron Microscopy," *Limnology and Oceanography*, vol. 43, no. 5, pp. 896–908.

Stagg, R. M. and T. J. Shuttleworth. 1982. "The Effects of Copper on Ionic Regulation by the Gills of Seawater-Adapted Flounder (*Platichthys flesus L.*)," *Journal of Comparative Physiology*, vol. 149B, pp. 83–90.

Seligman, P. F., A. O. Valkirs, J. S. Caso, I Rivera–Duarte, and E. Haslbeck. 2001. "Copper Release Rates from Antifouling Marine Coatings and Their Relationship to Loading and Toxicity in San

Diego Bay, CA," in *Proceedings of the Symposium on Pollution Prevention from Ships and Shipyards*, (M.A. Champ, ed.), Oceanology International Conference, 4–5 April 2001, Miami, Fl. pp. 64–81.

Tang, D. and P. H. Santschi. 2000. "Sensitive Determination of Dissolved Sulfide in Estuarine Water by Solid Phase Extraction and Hig-Performance Liquid Chromatography Detection of Methylene Blue," *Journal of Chromatography A*, vol. 883, pp. 305–309.

Tang, D., P. H. Santschi, C.-C. Hung, and K. Warnken. 2000a. "The Distribution of Biogenic Thiols in Surface Waters of Galveston Bay," *Limnology and Oceanography*, vol. 45, no. 6, pp. 1289–1297.

Tang, D., L.-S. Wen, and P. H. Santschi. 2000b. "Analysis of Biogenic Thiols in Natural Samples by HPLC Separation and Fluorescence Detection with Ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F)," *Analytica Chimica Acta,* vol. 408, pp. 299–307.

Tang, D., K. W. Warnken, and P. H. Santschi. 2001. "Organic Complexation of Copper in Surface Waters of Galveston Bay," *Limnology and Oceanography*, vol. 46, no. 2, pp. 321–330.

USEPA, October 28, 1999. "Notice of Intent to Revise Aquatic Life Criteria for Copper, Silver, Lead, Cadmium, Iron and Selenium," *Federal Register*, vol. 64, no. 209, Document 99-28314, pp. 58409–58410.

van den Berg, C. M. G. 1984. "Determination of the Complexing Capacity and Conditional Stability Constants of Complexes of Copper (II) with Natural Organic Ligands in Seawater by Cathodic Stripping Voltammetry of Copper-catechol Complex Ions," *Marine Chemistry*, vol. 15, pp. 1–18.

Webb, N. A. and C. M. Wood. 1998. "Physiological Analysis of the Stress Response Associated with Acute Silver Nitrate Exposure in Freshwater Rainbow Trout (*Oncorhynchus mykiss*)," *Environmental Toxicology and Chemistry*, vol. 17, no. 4, pp. 579–588.

Westall, J. J., F. M. M. Morel, and D. N. Hume. 1979. "Chloride Interference in Cupric Ion Selective Electrode Measurements," *Analytical Chemistry*, vol. 51, pp. 1792–1798.

Wilson, R. W. and E. W. Taylor, 1993a. "The Physiological Responses of Freshwater Rainbow Trout (*Oncorhynchus mykiss*) During Acutely Lethal Copper Exposure," *Journal of Comparative Physiology B*, vol. 163, pp. 38–47.

Wilson, R. W. and E. W. Taylor, 1993b. "Differential Responses to Copper in Rainbow Trout (*Oncorhynchus mykiss*) Acclimated to Sea Water and Brackish Water," *Journal of Comparative Physiology B*, vol. 163, pp. 239–246.

Wood, C. M., 1989. "The Physiological Problems of Fish In Acid Waters," in *Acid Toxicity and Aquatic Animals*, (R. Morris, E.W. Taylor, D.J.A. Brown, and J. A. Brown, eds.), *Society of Experimental Biology Seminar Series*, vol. 34, Cambridge University Press, Cambridge, U.K., pp. 125–152.

Wood, C. M., C. Hogstrand, F. Galvez, and R. S. Munger. 1996. "The Physiology of Waterborne Silver Toxicity in Freshwater Rainbow Trout (*Oncorhynchus mykiss*), 1. The Effects of Ionic Ag$^+$," *Aquatic Toxicology*, vol. 35, pp. 93–109.

Wood, C. M. 2001. "Toxic Responses of the Gill," Chapter 1 in *Target Organ Toxicity in Marine and Freshwater Teleosts, Volume 1—Organs*, (D. Schlenk and W. H. Benson, eds.), Taylor and Francis, London and New York, pp. 1–89.

Zirino, A., and S. Yamamoto. 1972. "A pH-Dependent Model for the Chemical Speciation of Copper, Zinc, Cadmium, and Lead in Seawater," *Limnology and Oceanography*, vol. 17, pp. 661–671.

Zirino, A., D. A. VanderWeele, S. L. Belli, R. De Marco, and D. J. Mackey. 1998a. "Direct Measurement of $Cu(II)_{aq}$ in Seawater with the Jalpaite Ion Selective Electrode," *Marine Chemistry*, vol. 61, pp. 173–184.

Zirino, A., S. L. Belli, and D. A. VanderWeele. 1998b. "Copper Activity and Copper Concentration in San Diego Bay," *Electroanalysis*, vol. 10, pp. 423–6427.

Zirino, A., R. De Marco, I. Rivera–Duarte, and B. Pejcic. 2002. "The Influence of Diffusion Fluxes on the Detection Limit of the Jalpaite Copper Ion-Selective Electrode," *Electroanalysis*, vol. 14, no. 7–8, pp. 493–498.

APPENDIX: COPPER WORKSHOP ATTENDEES

1 to 2 November 2000

NAME	Organization and Address	Phone/Fax	e-mail
*Allen, Herbert, PhD	Department of Civil and Environmental Engineering 354 Du Pont Hall University of Delaware Newark, DE 19716	302-831-8449 fax: 302-831-3640	allen@mail.ce.udel.edu
Benze, Robert	11055 Mountain Vista Circle NW Silverdale WA 98383	360-475-6929	rjbcjb@home.com
*Bruland, Ken, PhD	Institute of Marine Sciences University of California, Santa Cruz, Santa Cruz, CA 95064	408-459-4587 fax 408-459-4882	bruland@cats.ucsc.edu
Champ, Michael, PhD	ATRP Corporation PO Box 2439 7000 Vagabond Drive Falls Church, Virginia 22042-3934	703-237-0505 Fax 703-241-1278	MAChamp@aol.com
*De Marco, Roland, PhD	School of Applied Chemistry Curtin University GPO Box U 1987 Perth 6845 Western Australia	61 8 9266 3732 fax: +61 8 9266 2300	idemarco@info.curtin.edu.au
*Donat, John R., PhD	Department of Chemistry and Biochemistry Old Dominion University Norfolk, VA23529-0266	757-683-4098	jdonat@odu.edu
Dwyer, Bob, PhD	International Copper Assoc., Ltd. 260 Madison Avenue New York, NY 10016	212-251-7240	RDwyer@copper.org
*Gordon, Andrew, PhD	Dept. of Biological Sciences Old Dominion University Norfolk, VA23529-0266	757-683-3595 fax: 757-683-5283	agordon@odu.edu
Herrin, Russ	Water Chemistry Program U. Wisconsin-Madison 660 N. Park St. Madison WI 53706	608-265-6272	rherrin@facstaff.wisc.edu
*Moffett, James, PhD	Dept. of Marine Chemistry & Geo-Chemistry Woods Hole Oceanographic Inst. Woods Hole, MA 02543	408-289-3218	jmoffett@whoi.edu
*Paquin, Paul, PhD	HydroQual Inc. 1 Lethbridge Plaza Mahwah, NJ 07430	201-529-5151 x7144	ppaquin@hydroqual.com
Santore, R, PhD	HydroQual Inc. 1 Lethbridge Plaza Mahwah, NJ 07430	201-529-5151	
*Santschi, Peter, PhD	Dept. of Oceanography Texas A&M University 5007 Avenue U Galveston, TX 77551	409-740-4476	santschi@tamug.tamu.edu
*Shafer, Martin, PhD	Water Science and Engineering Laboratory University of Wisconsin Madison 660 North Park Madison, WI 53706	608-462-0140 fax: 608-262-0454	mmshafer@facstaff.wisc.edu

*Presenters

NAME	Organization and Address	Phone/Fax	e-mail
*Skrabal, Stephen, PhD	Department of Chemistry University of North Carolina – Wilmington 601 S. College Rd Wilmington, NC, 28403-3297	910-962-7160 fax: 910-962-3013	skrabals@uncwil.edu
*Thompson, Richard, PhD	School of Medicine University of Maryland		rthompso@umaryland.edu
*Weis, Peddrick, PhD	Dept. of Anatomy, NJ Medical School University of Medicine and Dentistry of New Jersey Newark, NJ 07103	973-972-4409	weis@umdnj.edu
E P A INVITEES			
*Delos, Charles	EPA OW/OST 4304 U.S. EPA Washington, DC 20460	202-260-7039 fax: 202-260-1036	delos.charles@epamail.epa.gov
*Mitchell, Jennifer	EPA OW/OST U.S. EPA Washington DC 20460	202-260-6101	mitchell.jennifer@epa.gov
Rappoli, Brian, PhD	U.S. EPA EPA/OW/OWOW/OCPD	202-260-9590	Rappoli.brian@epa.gov
Stapleton, Greg	EPA (OW) 4303 U.S. EPA Washington, DC 20460	202- 260-0141	stapleton.gregory@epamail.epa.gov
NAVY INVITEES			
*Beckwith, Bruce	PSNSY Code 106W Puget Sound Naval Shipyard Bremerton, WA 98314-5001	360-476-0118	beckwithb@psns.navy.mil
*Bohlander, Jerry	NSWCCD Code 6410 9500 MacArthur Blvd. W. Bethesda, MD 20817-5700	301-227-4498 fax: 301-227-4789	BohlanderGS@nswccd.navy.mil
Chrisey, Linda, PhD	Office of Naval Research(342) 9500 MacArthur Blvd. W. Bethesda, MD 20817-5700	703-696-4986	chrisel@onr.navy.mil
*Cotnoir, David	Atlantic Division NAVFACENGCOM(Code 1812) 1510 Gilbert St. Norfolk VA 23511	757-322-4733	Cotnoirdj@efdlant.navfac.navy.mil
Field, Andrew	NSWCCD Code 641 9500 MacArthur Blvd. W. Bethesda, MD 20817-5700	301-227-4783	fieldaj@nnowccd.navy.mil
*Haslbeck, Elizabeth	NSWCCD Code 641 Paints and Processes Division 9500 MacArthur Blvd. W. Bethesda, MD 20817-5700	301-227-4784	HaslbeckEG@nswccd.navy.mil
Holm, Eric, PhD	NSWCCD, Code 641 9500 MacArthur Blvd. W. Bethesda, MD 20817-5700	301-227-4948	HolmER@nswccd.navy.mil
Holst, Robert, PhD	SERDP/DUSD(ES) 901 N. Stuart ST. Suite 303 Arlington VA 22203-1853	703-696-2125	holstrw@acq.osd.mil
*Ingle, Mark	NAVSEASYSCOM(05M3) 1333 Issac Hull Ave Stop 5131 Washington Navy Yard DC 20376-5131	202-781-3665	inglemw@navsea.navy.mil

*Presenters

NAME	Organization and Address	Phone/Fax	e-mail
Lardis, Alex	Office of Naval Research (331) 800 N. Quincy St. Arlington, VA 22217-5660	301-227-4977 703-696-0989	LardisAE@nswccd.navy.mil lardisa@onr.navy.mil
McElvany, Stephan, PhD	Office of Naval Research (331) 800 N. Quincy St. Arlington, VA 22217-5660	703-696-1449	mcelvas@onr.navy.mil
*McCue, Tom	Naval Sea Systems Command (00C) 1333 Issac Hull Ave Stop 1070 Washington Navy Yard DC 20376-1070	703-607-2761	MccueTP@NAVSEA.NAVY.MIL
Montemarano, Jean	NSWCCD, Code 641 9500 MacArthur Blvd. Bethesda, MD 20817-5700	301-227-4964	MontemaranoJA@nswccd.navy.mil
Schatzberg, Paul	NACI 700 Melvin Ave, suite 9 Annapolis MD 21401	410-268-5391	PaulSchatzberg@cs.com
*Smith, Gordon	Naval Sea Systems Command (05M) 1333 Issac Hull Ave Stop 5121 Washington Navy Yard DC 20376-5121	202-781-3651	SmithG@navsea.navy.mil
SPAWAR Systems Center, San Diego PARTICIPANTS	Environmental Sciences Division		
*Chadwick, Bart, PhD	SSC SD Code 2362 53475 Strothe Road San Diego, CA 92152-6335	619-553-5333	chadwick@spawar.navy.mil
Earley, Pat	SSC SD Code 2362 53475 Strothe Road San Diego, CA 92152-6335	619-553-2768	earl@spawar.navy.mil
Grovhoug, Jeff	SSC SD Code 2362 53475 Strothe Road San Diego, CA 92152-6335	619-553-5475	grovhoug@spawar.navy.mil
*Rivera–Duarte, Ignacio	SSC SD Code 2361 53475 Strothe Road San Diego, CA 92152-6335	619-553-2373	iriverad@spawar.navy.mil
*Seligman, Peter	SSC SD Code 23601 53475 Strothe Road San Diego, CA 92152-6335	619-553-2760 fax 553-6553	seligman@spawar.navy.mil
Valkirs, Aldis	Computer Sciences Corp. 4045 Hancock St San Diego CA 92107	619-553-2770	aldis@spawar.navy.mil
*Zirino, Al, PhD	SSC SD Code 2361 53475 Strothe Road San Diego, CA 92152-6335	619-553-2772	zirino@spawar.navy.mil

*Presenters

REPORT DOCUMENTATION PAGE

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
08–2002	Final	

4. TITLE AND SUBTITLE

COPPER CHEMISTRY, TOXICITY, AND BIOAVAILABILITY AND ITS RELATIONSHIP TO REGULATION IN THE MARINE ENVIRONMENT

Office of Naval Research Second Workshop Report

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHORS

A. Zirino, ed.

P. F. Seligman, ed.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

SSC San Diego
San Diego, CA 92152–5001

8. PERFORMING ORGANIZATION REPORT NUMBER

TD 3140

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
800 North Quincy Street
Arlington, VA 22217–5660

10. SPONSOR/MONITOR'S ACRONYM(S)

ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

This is the work of the United States Government and therefore is not copyrighted. This work may be copied and disseminated without restriction. Many SSC San Diego public release documents are available in electronic format at http://www.spawar.navy.mil/sti/publications/pubs/index.html

14. ABSTRACT

This document details issues addressed at a November 2000 workshop attended by Navy and regulatory representatives and scientific experts. Objectives discussed were (1) define Navy copper compliance issues, problems, and requirements; (2) define current status and future direction of Cu regulations; (3) further the understanding of the relationship between copper speciation, bioavailability, and toxicity; (4) determine the state-of-the-science of the Cu free ion activity and biotic ligand models; and (5) develop a consensus on the maturity of free Cu ion activity and complexation capacity measurement capabilities.

15. SUBJECT TERMS

Mission Area: Navigation and Applied Sciences
environmental programs
analytical chemistry
marine biology
treatment and technologies

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			P. F. Seligman
U	U	U	UU	104	**19B. TELEPHONE NUMBER** *(Include area code)* (619) 553–5403

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

INITIAL DISTRIBUTION

20012	Patent Counsel	(1)
202753	Archive/Stock	(6)
202752	Library	(2)
2027	M. E. Cathcart	(1)
20275	E. R. Ratliff	(1)
202753	D. Richter	(1)
23601	P. Seligman	(50)
2361	M. Putnam	(1)
2362	J. Grovhoug	(2)
2362	S. Curtis	(1)
2362	R. Gauthier	(1)
2362	C. Katz	(1)
2362	D. Lapota	(1)
2362	J. Leather	(1)
2362	K. Richter	(1)
2362	P. F. Wang	(1)
2362	B. Wild	(1)
23621	S. Gagnon	(1)
23621	R. Fransham	(10)
23621	C. Johnson	(2)
23621	B. Johnston	(1)

Defense Technical Information Center
Fort Belvoir, VA 22060–6218 (4)

SSC San Diego Liaison Office
C/O PEO-SCS
Arlington, VA 22202–4804

Center for Naval Analyses
Alexandria, VA 22311–1850

Office of Naval Research
ATTN: NARDIC (Code 362)
Arlington, VA 22217–5660

Government-Industry Data Exchange
   Program Operations Center
Corona, CA 91718–8000

Office of Naval Research
Arlington, VA 22217–5660 (6)

Naval Sea Systems Command
Washington Navy Yard, DC 20376–1210 (4)

Chief of Naval Operations
Arlington, VA 22202–3735 (2)

Naval Facilities Engineering Command
Washington Navy Yard, DC 20374–5065 (3)

Commander Naval Region Southwest
San Diego, CA 92147–5110 (3)

Workshop Attendees (1) each